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(71) Applicant *(for all designated States except US)*: NATIONAL JEWISH CENTER FOR IMMUNOLOGY AND RESPIRATORY MEDICINE [US/US]; 1400 Jackson Street, Denver, CO 80206 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)* : KAPPLER, John, W. [US/US]; MARRACK, Philippa [US/US]; 4350 Montview Boulevard, Denver, CO 80207 (US).

(74) Agents: GREGG-EMERY, Valeta, A. et al.; Beaton & Swanson, 4582 S. Ulster Street Parkway, Suite 403, Denver, CO 80237 (US).

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(54) Title: PROTECTIVE EFFECTS OF MUTATED SUPERANTIGENS

(57) Abstract

The present invention includes a method for preventing or treating the toxic effects of a superantigen. A subject is treated with a molecule which interacts with specific Vβ elements of T cells in a manner similar to that of a native superantigen. The molecules of the present invention are mutated or modified superantigens which elicit antibody production without inducing T cell proliferation.

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PROTECTIVE EFFECTS OF MUTATED SUPERANTIGENS

FIELD OF THE INVENTION

This invention relates to methods for preventing and treating antigen-mediated and antigen-initiated diseases. Specifically, it relates to providing protection against superantigen pathogens by administration of molecules which are modified or mutated superantigens which elicit a antibody response against the superantigen without having the pathological effect of the superantigen. The molecules of this invention may also interact with the Vβ elements of T cell receptors in a way which leads to modifications in the way T cells respond to an antigen.

BACKGROUND OF THE INVENTION

The vertebrate immune system evolved to protect vertebrates from infection by microorganisms and large parasites. The immune system responds to antigens in one of two ways: (1) humoral antibody responses, mediated through B cells, involving the production of protein antibodies which circulate in the bloodstream and bind specifically to the foreign antigen which induced them. The binding of the antibody to the antigen makes it easier for phagocytic cells to ingest the antigen and often activates a system of blood proteins, collectively called complement, that helps destroy the antigen; and (2) cell-mediated immune responses, mediated through T cells, involving the production of specialized cells that react mainly with foreign antigens on the surface of host cells, either killing the host cell if the antigen is an infecting virus or inducing other host cells, such as macrophages, to destroy the antigen (Molecular Biology of the Cell (1983), B. Alberts et al. (eds), chapter 17, pp. 952).

The production of antibodies requires a number of preceding events to occur which lead to stimulation of

B cells producing the antibodies. One of the key events involved in the processes leading to antibody production is that of antigen recognition. Antigen recognition requires the participation of thymus (T) cells.

T cells have antigen-specific receptors on their surfaces, termed T cell antigen receptors (TCR). Before T cells can recognize protein antigens, the antigens must be presented on the surface of antigen-presenting cells. The antigens must first be processed by macrophages or other antigen presenting cells. These cells essentially swallow antigens and chop them into peptides which are displayed at the cell surface in combination with major histocompability complex (MHC) molecules.

The major histocompatibility antigens are a family of antigens encoded by a complex of genes called the major histocompatibility complex. MHC antigens are expressed on the cells of all higher vertebrates. In man they are called HLA antigens (human-leucocyte-associated antigens) because they were first demonstrated on leucocytes. There are two principal classes of MHC molecules, class I and class II, each comprising a set of cell-surface glycoproteins. The two classes of MHC antigens stimulate different subpopulations of T cells. MHC class II molecules are involved in most responses to pathogens. In contrast, MHC class I molecules are involved when the pathogen is a virus or when a malignant cell is involved. When MHC class I is involved, antibody stimulation does not result; rather, the interaction of MHC class I processed antigen and T-cell leads to lysis of cells infected with the pathogen.

The processed antigen peptide fits in a cleft on an MHC molecule. Once an antigen is displayed, the few T cells in the body that bear receptors for that particular peptide bind that complex. Most T cells

recognize antigens on the surface of cells only in association with self-MHC glycoproteins expressed on the same cell surface.

The ability of the T cell to complex with the processed antigen and MHC complex is dependent on the T cell receptor (TCR). The TCR consists of two protein chains, α and β. Each chain contains a constant and a variable domain. The variable domains are encoded in two (α) or three (β) different gene segments (variable (V), diversity (D), joining (J)) (Siu et al. (1984) Cell 37:393; Yanagi et al. (1985) Proc. Natl. Acad. Sci. USA 82:3430). In each T cell, the combination of V, D, and J domains of both the α and β chains participates in antigen recognition in a manner which is uniquely characteristic of that T cell and defines a unique binding site. See, Marrack et al. (1988) Immunol. Today 9:308; Toyonaga et al. (1987) Ann. Rev. Immunol. 5:585; Davis (1985) Ann. Rev. Immunol. 4:529; Hendrick et al. (1982) Cell 30:141; Babbitt et al. (1985) Nature 317:359; Buus et al. (1987) Science 235:1353; Townsend et al. (1986) Cell 44:959; Bjorkman et al. (1987) Nature 329:506). Generally, both the α and β chains are involved in recognition of the ligand formed by processed antigen and MHC.

When T cells are stimulated by an antigen, they divide and differentiate into activated effector cells that are responsible for various cell-mediated immune reactions. At least three different reactions are carried out by T cells: (1) cytotoxic T cells specifically kill foreign or virus-infected vertebrate cells; (2) helper T cells help B lymphocytes; and (3) suppressor T cells supress the responses of specific cells.

Recently, it has been shown that a novel class of antigens, termed "superantigens", are able to directly stimulate T cells by binding to a particular Vβ element, that is, the variable domain of the β chain of

the TCR (Kappler et al. (1987) Cell 49:263; Kappler et al. (1987) Cell 49:273; MacDonald et al. (1988) Nature 332:40; Pullen et al. (1988) Nature 335:796; Kappler et al. (1988) Nature 332:35; Abe et al. (1988) J. Immunol. 140:4132; White et al. (1989) Cell 56:27; Janeway et al. (1989) Immunol. Rev. 107:61; Berkoff et al. (1988) J. Immunol. 139:3189; Kappler et al. (1989) Science 244:811). Unlike recognition of conventional peptide antigens, the other components of the T cell receptor (i.e., Dβ, Jβ, Vα, Jα) appear to play little role in the superantigen binding. Superantigens, while generally stimulatory to T cells, appear to interact specifically with particular Vβ elements present on the stimulated T cell. Since the relative number of Vβ genes is limited, many T cells within an individual will bear a particular Vβ element, and a given superantigen is therefore capable of interacting with a large fraction of the T cell repertoire. Thus, depending on the frequency of the responding Vβ population(s), 5-30% of the entire T cell repertoire could be stimulated by a superantigen, whereas the responding frequency to a conventional antigen is usually much less than 1 in 1,000. Although superantigens interact with class II MHC molecules, they appear to act as intact proteins rather than as peptides, that is, they do not appear to bind within the conventional peptide binding groove. Instead, they seem to interact with amino acid residues that are on the outer walls of the binding cleft. Known superantigens and references to their sequences and structures are listed in Table I.

Two distinct classes of superantigen have been described. The first was noted nearly 20 years ago, when Festenstein showed marked responses in mixed lymphocyte reactions between certain MHC identical strains. The stimulating antigens were called minor lymphocyte stimulating (Mls) antigens (Festenstein

(1973) Transplant Rev. 15:62) to differentiate them from MHC antigens. These superantigens are encoded by endogenous retroviral genes (Palmer (1991) Curr. Bio. 1:74). The presence of these genes in the mouse leads to a marked deletion of responding T cells, creating potentially large holes in the animal's T cell receptor repertoire (Pullen et al. (1988) supra). The second set of superantigen is represented by a growing list of bacterial and viral proteins, capable of producing a variety of pathological effects after injection (Marrack & Kappler (1990) Science 248:705).

Staphylococcus aureus (S. aureus), a common human pathogen, produces several enterotoxins, designated as SEA (staphylococcal enterotoxin A) through SEE, which can be responsible for food poisoning and occasionally shock in humans (Marrack & Kappler (1990) supra; Bohach et al. (1990) Crit. Rev. Microbio. 117:251). Some S. aureus isolates also produce toxic shock syndrome toxin-1 (TSST-1), which has been implicated in the majority of cases of human toxic shock syndrome as well as the related exfoliative toxins (ExTF), which are associated with the scalded skin syndrome. Streptococcus pyrogenes, or group A streptococcus, another common human pathogen of the skin and pharynx, also produces toxins with superantigenic properties (Abe et al. (1991) J. Immun. 146:3747). These have been designated streptococcal erythrogenic toxins A-C (SPEA-C).

The amino acid sequence of the S. aureus toxins exhibit some homology, but also exhibit marked differences (See, Bentley et al. (1988) J. Bacteriol. 170:34; Jones et al. (1986) J. Bacteriol. 166:29; Lee et al. (1988) J. Bacteriol. 170:2954; Blomster-Hautamaa et al. (1986) J. Biol. Chem. 261:15783). S. aureus has the ability to stimulate powerful T cell proliferation responses in the presence of mouse cells bearing MHC class II type molecules (Carlson et al.

(1988) J. Immunol. 140:2848; White et al. (1989) Cell 56:27). The S. aureus proteins selectively stimulate murine cells bearing particular Vβ elements.

The binding of toxins to class II MHC molecules is a prerequisite for T cell recognition, but the process is much more permissive for superantigens than that seen with conventional antigens. While peptide antigens are very dependent on allelic MHC residues for binding, the superantigens bind to a wide variety of allelic and isotypic forms of MHC class II molecules (See, Hermann et al. (1989) Eur. J. Immunol. 19:2171; Herman et al. (1990) J. Exp. Med. 172:709; Scholl et al. (1990) J. Immunol. 144:226; Molleck et al. (1991) J. Immunol. 146:463). While T cells rarely recognize peptide antigens bound to self-MHC (allo-MHC) molecules, individual T cell clones can respond to toxins bound not only to various allelic forms of MHC, but also to different class II isotypes and even to xenogenic class II molecules. Such observations suggest that superantigens bind at a relatively conserved site outside the allelically hypervariable groove thought to bind conventional peptide antigens.

Superantigens may contribute to autoimmune diseases, in which components of the immune system attack normal tissue. The process of deletion of T cells responsive to self, potentially harmful self-reactive T cells, is called tolerance or negative selection (Kappler et al. (1987) Cell 49:273; Kapper et al. (1988) Nature 332:35; MacDonald et al. (1988) Nature 332:40; Finkel et al. (1989) Cell 58:1047). The immune system normally deletes self-reactive T cells, but occasionally a few appear to escape the surveillance mechanism. It has been suggested that the ability of superantigens to arouse 20 percent of a person's T cell repertoire could lead to undesirable replication of the few circulating T cells that recognize self (Johnson et al. (1992) Scientific

American 266:92). T cells bearing certain Vβ types have been implicated in various autoimmune conditions, including arthritis and multiple sclerosis. These findings imply that the destructive cells might be activated by a superantigen that binds to the identified Vβ types (Johnson et al. (1992) supra).

Autoimmune diseases are a result of a failure of the immune system to avoid recognition of self. The attack by the immune system of host cells can result in a large number of disorders, including such neural diseases as multiple sclerosis and myasthenia gravis, diseases of the joints, such as rheumatoid arthritis, attacks on nucleic acids, as observed with systemic lupus erythematosus, and such other diseases associated with various organs, as psoriasis, juvenile onset diabetes, Sjögren's disease, and thyroid disease. These diseases can have a variety of symptoms, which can vary from minor and irritating to life-threatening. For example, rheumatoid arthritis (RA) is a chronic, recurrent inflammatory disease primarily involving joints, and affects 1-3% of North Americans, with a female to male ration of 3:1. Severe RA patients tend to exhibit extra-articular manisfestations including vasculitis, muscle atrophy, subcutaneous nodules, lymphadenopathy, splenomegaly, and leukopenia. It is estimated that about 15% of RA patients become completely incapacitated.

Several lines of evidence suggest that T cells specific for self-antigens may play a critical role in the initiation of autoimmune diseases. In the case of RA, the linkage of the disease to the DR4 and DR1 alleles of the class II genes of MHC and the findings that sometimes oligoclonal, activated $CD4^+$ T cells in synovial fluid and tissue of affected joints (Stastny et al. (1976) Engl. J. Med. 298:869; Gibofsky et al. (1978) J. Exp. Med. 148:1728; McMichael et al. (1977) Arth. Rheum. 20:1037; Schiff et al. (1982) Ann. Rheum.

Dis. 41:403; Duquestoy et al. (1984) Hum. Immunol. 10:165; Legrand et al. (1984) Am. J. Hum. Genet. 36:690; Gregerse et al. (1987) Arth. Rheum. 32:15; Burmester et al. (1981) Arth. Rheum. 24:1370; Fox et al. (1982) J. Immunol. 128:351; Hemler et al. (1986) J. Clin. Invest. 78:696; Stamenkoic et al. (1988) Proc. Natl. Acad. Sci. USA 85:1179) suggest the involvement of $CD4^+$, $\alpha\beta$TCR-bearing, class II-restricted T cells in the disease. This view is supported by the finding that partial elimination or inhibition of T cells by a variety of techniques can lead to an amelioration of disease in certain patients (Paulus et al. (1977) Arth. Rheum. 20:1249; Karsh et al. (1979) Arth. Rheum. 22:1055; Kotzin et al. (1989) N. Eng. J. Med. 305:976; Herzog et al. (1987) Lancet ii:1461; Yocum et al. (1989) Ann. Int. Med. 109:863).

U. S. patent application Serial No. 07/732,114, herein specifically incorporated by reference, establishes that specific Vβ elements may be used to diagnose for an autoimmune disease, specifically the presence of a higher percentage of $V\beta14^+$ T cells in synovial fluid may be used to diagnose RA.

Many investigative efforts have focused on developing methods for the treatment of autoimmune diseases. For example, European Patent Publication 340 109, entitled Anti-T-cell receptor determinants as autoimuune disease treatment, and U.S. Patent No. 4,550,086, issued October 29, 1985 to Reinherz et al., entitled Monoclonal antibodies that recognize human T cells, describe a method of detecting a particular sequence of the variable region gene of T cell receptors associated with a particular disease and treating the disease with antibodies to that sequence. U.S. Patent No. 4,886,743, issued December 12, 1989 to Hood et al., entitled: Diagnostic reagents based on unqiue sequences within the variable region of the T cell receptor and uses thereof, describes a method of

diagnosis diseases based on the presence of T cells with a unique sequence in the Vβ region associated with a specific disease. PCT Patent Application Publication WO 90/06758 describes a method for detecting specific Vβ regions associated with RA, specifically, Vβ3, Vβ9, and Vβ10, and for the treatment of RA with monoclonal antibodies which recognize Vβ3, Vβ9, and Vβ10.

Immunity

An animal that has never been exposed to a pathogen has no specific defenses against it. However, the animal can be immunized against the pathogen by injecting it with a non-virulent form of the pathogen with a similar chemical structure as the pathogen but without the ability to cause the pathological effect. The animal will produce antibodies specific against the non-virulent form of the pathogen, and these antibodies can protect the animal against attack from the virulent pathogen.

BRIEF SUMMARY OF THE INVENTION

The present invention includes a method for preventing the toxic effects of a superantigen by treatment with a molecule, wherein said molecule elicits antibody production without inducing T cell activation.

The present invention also includes molecules consisting of mutated or modified derivatives of superantigens.

The present invention further includes a method of modifying T cell response elicited by an antigen comprising administering a molecule which interacts with either the Vβ element alone or both the α and β chains of T cell receptors (TCR).

The molecules of this invention can function by leading to deletion or inactivation/desensitization of

at least one or more subpopulations of T cells which present a particular Vβ element.

To prevent the in vivo toxic effect of superantigen requires an exact understanding of how their effect is achieved. Prior to this invention, while it was known how superantigen interact with T cells, the manner in which a subject animal developed a pathological condition and whether a pathological condition would develop was not well understood. Various observations suggested that any of a number of mechanisms could be the cause of the toxicity.

It has now been found that the pathological condition mediated or initiated by a superantigen can be prevented or treated by administration of the mutant superantigen molecules of the present invention. Administration of the mutant toxins of the present invention may cause antibody production against the mutant molecule. Some of these antibodies also react with the normal non-mutated toxin. Therefore, when the immunized individual is confronted with the normal toxin, these cross-reactive antibodies react with the normal toxin and inhibit its toxic activity.

BRIEF DESCRIPTION OF THE FIGURES

FIGURE 1 is a schematic ribbon drawing of the three dimensional structure of SEB. Region 1 (residues 9-23), region 2 (residues 40-53), and region 3 (residues 60-61) are differentiated by shading. Sites identified as involved in MHC or TCR binding are shown. Residues identified by mutational analysis as important to MHC and/or TCR binding are indicated.

FIGURE 2a shows the SDS-PAGE analysis of 2 ug of recombinant SEB purified from E. coli and wild-type SEB purified from S. aureus cultures. Molecular mass markers (in kD): β-phosphorylase, 94; bovine albumin,

69; ovalbumin, 45; carboxylase, 30; soybean trypsin inhibitor, 21; lysozyme, 14. FIGURE 2b shows the SDS-PAGE analysis of SEB binding to DR on LG2 cells. Molecular mass markers (in kD): bovine albumin, 69; ovalbumin, 45; chymotrypsinogen, 27; soybean trypsin inhibitor, 21; myoglobin, 17; lysozyme, 14.

FIGURE 3 shows the sequences of the SEB mutants. Dashed lines indicate identity to unmutated SEB. The positions of the oligonucleotides used to generate the SEB mutants are also shown.

FIGURE 4 shows the binding of SEB and SEB mutants to DR1-bearing lymphoblastoid line LG2 cells.

FIGURE 5 shows stimulation of T cell hybridomas by region 1 SEB mutants. Preparations of purified SEB or the region 1 mutants were tested for their ability to stimulate a collection of T cell hybridomas bearing of the the Vβ elements known to recognize SEB: KS-20.15 (Vβ7), KS-6.1 (Vβ8.2), KS-47.1 (Vβ8.3), K16-57 (Vβ8.1).

FIGURE 6 shows stimulation of T cell hybridomas by region 2 SEB mutants. Preparations of purified SEB were tested as in Figure 5.

FIGURE 7 shows stimulation of T cell hybridomas by region 3 SEB mutants. Preparations of purified SEB were tested as in Figure 5.

FIGURE 8 shows the effects of SEB and its mutants in vivo. Groups of three mice were weighed and then given balanced salt solution (BSS) containing nothing, 50 ug (left), or 100 ug (right) of recombinant SEB or the mutant SEBs BR-257 or BR-358.

FIGURE 9 shows the protective effect of mutant toxins against challenge with SEB. Mice received doses of either saline or BR-257 three months prior to challenge with wild-type SEB.

DETAILED DESCRIPTION OF THE INVENTION

The molecules of the present invention may be effective in different ways in preventing or treating antigen-mediated or initiated diseases. Some of the different ways in which the molecules of the present invention may be effective include modification of the T cell response and production of antibodies which provide protection against pathogens. Specifically, this invention presents a method of preventing or treating superantigen-mediated or superantigen initiated diseases. The method of this invention generally involves preparing mutated superantigen molecules by methods known in the art and described herein, identifying antigen mutants unable to bind either MHC or TCR, and testing for ability to protect against exposure to the non-mutated superantigen.

The present invention describes the feasibility of the above-outlined approach in achieving protection against a known superantigen. Mutants of recombinant Staphylococcal enterotoxin B (SEB) were prepared and purified as described in Examples 1-4 below. SEB mutants unable to bind MHC molecules or TCR were selected by examining the binding of mutant SEB molecules to HLA-DR1 homozygous lymphoblastoid line LG2 cells and stimulation of T cell hybridomas bearing different Vβ elements. The SEB mutant BR-257, which bound LG2 cells in a manner indistinguishable from that of non-mutated SEB and did not stimulate T cell hybridomas, injected into experimental animals 3 months prior to exposure to SEB provided complete protection against the toxic effects of SEB. Similar results were obtained in primates.

Although the present disclosure describes production of mutated SEB molecules able to protect animals against subsequent challenge with SEB, the methods of the present invention are equally applicable to other superantigens.

The ability to use the occurrence of specific Vβ elements to diagnose autoimmune diseases, as discussed in detail above, may be combined with the present invention as a method of detecting and treating autoimmune diseases mediated by superantigens. The existence of a superantigen-mediated disease may be determined by a "footprint" analysis, e.g., by determining if there is an alteration in Vβ elements in a disease state. The finding of alterations in Vβ elements, such as the increase in Vβ14$^+$ T cells in synovial fluid in RA, suggests the presence of a superantigen-mediated disease. Techniques known to the art may then be applied in order to isolate and identify the implicated superantigen. The Vβ footprint may be compared against that of a known superantigen for possible implication of that superantigen in initiation or proliferation of the disease. There may a search for genes coding for a superantigen when a virus or bacterial infection is associated with the initiation of the disease. Once a superantigen is identified or isolated, the method of the present invention may be applied to produce a mutant superantigen molecule capable of conferring protection against exposure to the superantigen.

Various terms are used in this specification, for which it may be helpful to have definitions. These are provided herein, and should be borne in mind when these terms are used in the following examples.

As described above, the key event in an immune response is the interaction of MHC molecules with antigens to form a complex presented to T cells. Generally, the T cell response is quite specific, with

only very limited subpopulations of T cells responding to specific complexes of antigen and MHC molecules. The response generally requires interaction of most or all of the components of the T cell receptor. In certain circumstances, however, the presented antigen need only interact with the Vβ element of the receptor, all other components are essentially irrelevant. This means that the antigen can, and does, react with a much greater array of T cells than is normally the case.

The molecules of this invention may interact with the Vβ elements of T cell receptors in a way which leads to modifications in the way T cells respond to a superantigen. "Modifying T cell responsiveness" means that the mutant molecules are able to change the manner in which the subject's T cells respond when provoked by the administered molecule, or to an antigen administered concurrently, previously, or afterward. For example, it is believed that early in the development of T cells, certain subpopulations interact with presented antigens and are deleted. The molecules of this invention can function in this manner, i.e., by leading to deletion or inactivation/desensitization of at least one or more subpopulations of T cells which present a particular Vβ element.

In a particular embodiment of the present invention, the molecules modify the T cell response without changing the B cell response that would normally occur in the subject under consideration. This type of material is useful, for example, for providing passive immunity to a subject, or serving as a vaccine. When superantigen derivatives are used, these derivatives are no longer superantigenic, as they will not provoke a restricted T cell response, but will still serve as antigens in that they generate a B cell response. The superantigen derivatives of the present invention are able to elicit normal antibody production against the superantigen protein.

The molecules of the present invention may also be seen as competitors for other antigens. If the molecules described herein interact with MHC elements otherwise required for generation of a full scale response to an antigen or superantigen, they may prevent or reduce the extent of that response.

The molecules of the present invention may also be viewed as "enhancers" in some instances, where an individual's T cell responsiveness is impaired or weakened by any of a number of causes. Via administration of the molecules encompassed by the present invention, the T cell populations of the individual can be greatly expanded.

The term "modifying T cell responsiveness" as used herein is always relative to a second element (e.g., an antigen), and always refers in particular to responsiveness of T cells presenting a particular Vβ element as part of their T cell receptors, other components of the receptors being essentially irrelevant.

The molecules of the present invention contain, at least, an amino acid sequence of sufficient size to bind to an MHC molecule. The rest of the molecule may consist of amino acids, or may contain carbohydrate or lipid structures.

"Reducing responsiveness" is construed to also include deleting the portion of T cells expressing a particular Vβ element.

"Superantigen derivative" as used herein refers to a molecule whose structure, at the least, contains an amino acid sequence substantially identical to an amino acid sequence presented by a superantigen or portions of a superantigen required for binding to either the MHC or the T cell.

"Modified" superantigen derivative (or fragment), differs from "mutated" superantigen derivative (or fragment). The term "modified superantigen" is defined

to refer to molecules which contain an amino acid sequence identical to an amino acid sequence of superantigen, but contain modifications not found in the superantigen molecule itself. For example, if a superantigen contains amino acids 1-250, a "modified" superantigen derivative may contain a sequence identical to amino acids 50-75, positioned in between stretches of amino acids not found in the native superantigen molecule. Additional modifications may include, for example, differing or absent glycosylation patterns, or glycosylation where there normally is none.

"Mutated" superantigen refer to structures where the actual amino acid sequence of the mutation has been altered relative to the native form of the molecule. For example, if a superantigen contains amino acids 1-250, a mutated superantigen may include amino acids 50-68 and 72-75 which are identical to the corresponding native sequence, but differ in amino acids 69-71. The difference may be one of "substitution" where different amino acids are used, "addition" where more amino acids are included so that the sequence is longer than the native form, or "deletion" where the amino acids are missing.

"Vaccine" refers to a formulation when administered to a subject provokes the same type of response typical of vaccines in general, e.g., active immunological prophylaxis. The vaccine may contain adjuvant, or other materials.

It is known that the class of molecules known as superantigens interact with particular Vβ regions of T cell receptors, leading to massive proliferation of particular T cell subpopulations. This interaction, which assumes prior interaction between an MHC molecule and the superantigen, is almost completely independent of any other region of the T cell receptor.

In connection with the interaction of MHC and peptide, it must be noted that MHC molecules are available in a variety of "phenotypes", and different phenotypes are specific for various presented peptides and antigens. For example, HLA-DR is known to be associated with presentation of SEB. Thus, different MHC phenotypes will be of value for different antigens, but determination of HLA phenotype and correlation to presentation of a particular antigen or antigen family is well within the skill of the artisan in this field.

Thus, this invention involves the modification of the T cell response via administration to a subject of a molecule which interacts with both an MHC molecule and at least one Vβ element on T cell receptors. This interaction may affect the T cell response in any number of ways. Perhaps the most elementary manner of affecting the response is one where a molecule interacts with the MHC molecule, preventing the binding of other molecules to the MHC. If the competing molecule has been modified or does not naturally provoke proliferation of T cells, then there will be a lessening or elimination of the response because molecules such as normal antigens or superantigens cannot form the requisite complex with the MHC to generate a T cell proliferative response.

Another manner of modifying the T cell response is via "desensitizing", "inactivating", or "anergizing" the T cells. This mechanism involves interaction of MHC molecule, antigen, and T cell receptor, with subsequent down regulation or inactivation of the T cells. This mechanism is more common in mature subjects than the deletion phenomenon, which occurs in fetal subjects. The latter phenomenon is one where via interaction of the three units, various subpopulations of T cells are in fact removed from the organism.

The modification of the T cell response can also involve stimulation of T cell subpopulations.

Knowledge of the mechanisms described herein permits the artisan to administer to a subject a material which interacts with the MHC and a particular subpopulation of T cells, where proliferation of the T cell subpopulation results. This approach is particularly desirable in the treatment of conditions where a particular Vβ subpopulation or subpopulations are associated with a pathological condition, such as an autoimmune disease.

It should be understood that an immune response, when fully considered, includes both a B cell and a T cell response. One aspect of the invention involves the use of molecules which modify the T cell response without modifying the B cell response. Such materials are especially useful as vaccines, as discussed below.

The molecules of the invention are preferably, but not exclusively, superantigen derivatives. These derivatives may be modified or mutated, as discussed above. These, or any other molecules used herein, are administered in an amount sufficient to modify the T cell response in the manner described. The amount of material used will vary, depending on the actual material, the response desired, and the subject matter of the treatment.

The molecules may also serve as vaccines. These vaccines confer protective immunity on the subject in that they generate a B cell response without the full T cell response normally associated with the normal form of the molecule. Example 7 shows one manifestation of this effect for SEB. Again, depending upon the parameters within the control of the knowledge of the artisan, including the condition being treated, the Vβ molecule to be regulated, and so forth, the material chosen for the vaccine is up to the artisan. The vaccine may contain other materials which are normally found in vaccine compositions, including adjuvants, carriers, etc.

The mode of administration of the materials described herein may vary as well, including intravenous, intraperitoneal, and intramuscular injections, as well as all of the other standard methods for administering therapeutic agents to a subject.

The invention also discloses how to make particular mutants useful in the foregoing methodologies, including isolated nucleic acid sequences coding for mutants, cell lines transformed by these and the vectors and plasmids used therefor, as well as the isolated mutant molecules, including mutant superantigens.

Other applications of the invention described herein will be apparent to the skilled artisan and need not be repeated here.

The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, it being recognized that various modifications are possible within the scope of the invention.

Polymerase Chain Reaction (PCR) and standard molecular biological methodologies, described in Example 1, were used in the construction and expression of recombinant SEB. SEB mutants were generated in one of two ways, as described in Example 2. The first way introduced random mutations along the entire length of the SEB gene. A second method introduced random mutations in approximately 60-75 base-defined regions of the SEB gene. Initial identification of potential mutant SEBs tested the lysate from transformants for the presence of functional toxin by stimulation of murine T cell hybridomas bearing different Vβ elements in a human DR-expressing cell line. Lysates negative for T cell hybridoma stimulations were tested for the

presence of SEB with the use of monoclonal antibodies (mAbs) against SEB (Example 3). Transformants producing non-functional SEB were sequenced and the mutation identified. Transformants producing mutant SEBs were grown, mutant SEBs purified as described in Example 4. Analysis of the location and effect of the mutation was performed. Since binding to MHC class II molecules is a prerequisite for toxin recognition by T cells, the ability of mutant SEBs to bind human MHC antigen HLA-DR1 was tested as described in Example 5. Mutant SEBs, such as region 3 mutants, were produced which selectively stimulate some, but not all, of the hybridomas bearing specific Vβ elements stimulated by the non-mutated toxin. Thus, the mutated superantigens of the present invention may be used to selectively stimulate only some of the T cell populations stimulated by the wild-type superantigen.

Three regions were identified in the N-terminus part of SEB that affect MHC and/or TCR binding (Example 4 and Figure 1). Mutations in region 1 (residues 9-23) affected both MHC and TCR binding. The results suggested that 23N was particularly important. When the sequences of the S. aureus enterotoxins are aligned for maximum homology (Marrack & Kapple (1990) supra), this residue is conserved among all of the enterotoxins and toxic shock toxin as well. The mutations in region 2 (residues 41-53) drastically reduced the ability of the toxin to bind to MHC class II with a similar effect on their ability to stimulate T cells. About half of the mutations involved F44. Again, this residue is conserved among all the enterotoxins, indicating that this residue probably plays a critical role in the binding of all of the toxins to MHC. Interestingly, none of the mutations in either region 1 or 2 completely obliterated toxin binding to MHC, and in both cases the T cell-stimulating ability of the mutants could be recovered by adding a large excess of

toxin. Mutations in region 3 (60N, 61Y) did not affect binding of the toxins to MHC, but did affect their interaction with two Vβs, 7 and 8.1. This Vβ-specific effect suggests that these amino acids are important for interaction with some, but perhaps not other, TCR Vβs.

The toxicity of mutant SEBs in animals was tested as described in Example 6. Mice were injected with either balanced salt solution (BSS), recombinant SEB, or region 1 SEB mutants at F44 (BR-358) or at N23 (BR-257). Mice receiving 50 ug of either mutant SEB were indistinguishable from those receiving BSS, while those receiving recombinant SEB died within 5 days.

The ability of mutant SEB to provide immune protection from SEB was tested in vivo (Example 7). Mice receiving 100 ug of mutant SEB BR-257 three months prior to challenge with SEB were fully protected from the toxic effect of SEB, whereas those animals not injected with BR-257 died 4-5 days after challenge with SEB. Similar results were obtained with primates. Mutant SEB BR-358 and BR-257 were either ineffective or much less effective in eliciting an emetic response in monkeys (Example 7).

Example 8 describes the application of the above procedure to the SEA toxin and the production of a SEA mutant which behaves the same as the corresponding SEB mutant.

Example 1. Construction and Expression of Recombinant SEB.

Polymerase Chain Reaction (PCR). PCRs (Saiki et al. (1988) Science 239:487) were performed using AmpliTaq recombinant Taq polymerase and the DNA Thermal Cycler from Perkin Elmer Cetus (Norwalk, CT). 20-30 cycles were performed with 1-min denaturing and annealing steps, and an extension step of 1 min for synthesis < 500 bp and 2 min for those > 500 bp.

Template concentrations were 1-10 nM and oligonucleotides primer concentrations were 1 uM. The concentration of the dNTPs was 200 uM, except when attempting to introduce mutations, where the concentration of one of the dNTPs was reduced to 20 mM.

SEB Construct. The gene for superantigen SEB was overexpressed in E. coli as follows. A linearized plasmid containing the genomic SEB gene (Ranelli et al. (1985) Proc. Natl. Acad. Sci. USA 82:5850) was used as a template in a PCR utilizing oligonucleotide primers that flanked the portion of the gene encoding the mature SEB without the signal peptide. The 5′ primer was (SEQ ID NO:1):

5′-TAGGGAATTCCATGGAGAGTCAACCAGA-3′

This primer contains an EcoRI site which places the SEB gene in-frame with the LacZ gene when cloned into plasmid pTZ18R (Pharmacia Fine Chemicals, Piscataway, NJ). This oligonucleotide primer also contains an NcoI site which adds an ATG between the LacZ gene fragment and the beginning of the SEB gene so that the SEB gene could be moved easily to other plasmids carrying its own initiation ATG. The 3′ primer contained a HindIII site after the termination codon of the SEB gene (SEQ ID NO:2):

5′-AGCTAAGCTTCACTTTTTCTTTGTCG-3′

The PCR fragment was digested with EcoRI and HindIII and ligated into EcoRI/HindIII-digested pTZ18R. E. coli XL1-Blue (Stratagen, La Jolla, CA) was transformed with the plasmid, a single transformant picked, and the insert (pSEB2) was sequenced to check that it had no mutations.

Upon induction the pSEB2 construct led to overproduction of mostly cytoplasmic SEB (≈ 10 ug/ml of

broth). However, rather than producing a LacZ/SEB fusion protein, the bacteria produced a protein with the same apparent molecular weight as secreted SEB from S. aureus (Fig. 1a). Either the LacZ portion of the fusion protein was cleaved in vivo from the majority of SEB or the ATG introduced between LacZ and SEB was a more efficient translation initiation site than that of LacZ.

Example 2. Generation of SEB Mutants.

SEB mutants were generated in one of two ways. One way introduced random mutations along the entire length of the SEB gene. To do so, the SEB construct of Example 1 was prepared but PCR was performed with concentrations of either dATP or dTTP reduced 10 fold in order to increase Taq polymerase error rate (Innis et al. (1988) Proc. Natl. Acad. Sci. USA 85:9436). This reduces the product amount 5-10 fold. Products of the two reactions were combined, cloned into pTZ18R as described in Example 1, and individual transformants were screened for mutant SEB as described in Example 3 for BR mutants. Of approximately 400 toxin-producing transformants screened, 10 were identified as functional mutants by their reduced ability to stimulate T cells. Low concentrations of dCTP and dGTP were tried as well, but less reduction in product results and no mutants were detected in screening approximately 200 transformants.

A second PCR method was used for introducing random mutations in approximately 60-75 base-defined regions of the SEB gene. The following oligonucleotide primers (A (SEQ ID NO:3), B (SEQ ID NO:4), and C (SEQ ID NO:5)) positioned as shown in Figure 2, were synthesized with each position containing 1% each of the three incorrect bases:

A-: 5'ATTCCCTAACTTAGTGTCCTTAATAGAATATATTAAGTCAAAGTATAG AAATTGATCTATAGA3'

B-: 5'AGCTAGATCTTTGTTTTTAAATTCGACTCGAACATTATCATAATTCCC GAGCTTA3'

C+: 5'CCGGATCCTAAACCAGATGAGCTCCACAAATCTTCCAATTCACAGGCC TGATGGAAAATATGAAAGTTTGTAT3'

These mutant oligonucleotides served as primers in a PCR reaction with either a vector (A and B) or internal SEB (c) oligonucleotide as the other primer, and the SEB gene as the template. Each molecule of synthesized SEB fragment was predicted to have 2-3 random base mutations in the region corresponding to mutant primer. Mutant fragments were incorporated into the SEB gene, either alone or with another fragment containing the 3'-portion of the gene as mixed template in a PCR reaction to resynthesized a full length SEB2 gene (Ho et al. (1989) Gene 77:51; Pullen et al. (1990) Cell 61:1365). Alternatively, this was accomplished by digestion with appropriate restriction enzymes and ligation into pSEB2 from which the corresponding region had been removed.

DNA Sequencing. Plasmid inserts were sequenced directly by the dideoxynucleotide method of Sanger et al. (1977) Proc. Natl. Acad. Sci. USA 74:5463, using Sequenase (U.S. Biochemical Corp., Cleveland, OH) and a modification for double-stranded supercoiled plasmid templates (Weickert and Chambliss (1989) in Editorial Comments, U. S. Biochemical Corp., Cleveland, OH, pg. 5-6.

Example 3. Screening of Transformants for SEB Mutants.

Anti-SEB Monoclonal Antibodies (mAbs). 10 mAbs specific for at least five epitopes of SEB were produced by standard methods from B10.Q(βBR) immunized multiple times with SEB. One of these antibodies, B344.1, was used both for quantitation and immunoaffinity purification of SEB and SEB mutants. B344.1 is an IgG1 that was chosen because initial

characterization showed that it had a high affinity for SEB, bound equally well to all of the SEB functional mutants, could detect and immunoprecipitate SEB bound to MHC class II molecules, and did not block T cell recognition of SEB bound to DR (data not shown).

ELISA for SEB. The amount of SEB in preparations wad determined by ELISA. Microtiter wells were coated overnight with a solution of 6 ug/ml natural SEB (Sigma Chemical Co., St. Louis, MO). The wells were then incubated with 25% FCS and washed throughly. Various concentrations of known and unknown SEB preparations were added to the wells as inhibitor followed by a constant amount of anti-SEB antibody (polyclonal rabbit anti-SEB(Toxin Technology, Madison, WI) in BR experiments and monoclonal anti-SEB, B344, in BA, BB, and BC experiments). After 1 hour, the wells were washed thoroughly, and the bound antibody was detected by standard techniques using alkaline phosphatase coupling either to goat anti-rabbit IgG (Sigma Chemical Co.) or to p-nitrophenyl phosphate as substrate. The OD of the reaction at 405 nm was related to the dose of inhibitor and the concentration of the SEB in the unknown estimated by computer analysis of the data.

Initial Screening of Potentially Mutant SEB. For primary screening, totaly lysates were prepared as described from individual transformants containing a potentially mutant SEB gene. Aliquots of each lysate were tested for the presence of functional toxin by stimulation of murine T cell hybridomas bearing α/β receptors with either Vβ7 or Vβ8.3, using human DR-expressing cell lines as presenting cells. Lysates deficient in stimulating either of these hybridomas were assayed for the presence of SEB protein to rule out mutations affecting the level or the full length of the SEB produced. Plasmids from those producing proteins were sequenced to locate the mutation the sequences of the mutants are shown in Figure 2.

The Taq polymerase error-induced random mutants (BR) were clustered in three regions (1, 2, 4), all in the NH_2-terminal 93 amino acids of the molecule (except an additional conservative mutation in one case, BR-374, in the COOH-terminal half of the molecule). As predicted by their method of generation, all but one of these mutations involved a nucleotide substitution of A to G or T to C, and only one silent mutation was found elsewhere in their sequences (data not shown). Additional mutants were generated in region 1 or 2 with mutant oligonucleotide C or A (BC, BA mutants). Region 3 was originally discovered as a single mutant (BA-62) involving the last amino acid covered by oligonucleotide A. The mutant had a different phenotype than the other BA mutants. Additional mutants were produced in this region with mutant oligonucleotide B (BB mutants). Mutations in region 4 were eliminated from further analysis, because it was felt that interfering with the conserved disulfide forming cysteine at position 93 could have far reaching unpredictable effects. In addition, several mutants were not further characterized either because they involved more than one region (Br-474, BA-72), produced highly degraded toxin (BR-267), or were identical to an already existing mutant (BA-50).

Example 4. Preparation of Recombinant SEB.

For initial screening, individual colonies of transformants picked from agar plates were transferred to wells of 96-well microtiter plates containing 100 ul of 2XYT and carbenicillin. A replicate plate was prepared except that the media contained 1 mM IPTG as well. Both were incubated overnight at 37°C with shaking. 50 ul of glycerol was added to each well of the first plate, which was mixed and then stored at -70°C. To prepare SEB-containing lysates, each well of the second plate received 50 ul of HNM buffer (10 mM

Hepes, pH 7.0, 30 mM NaCl, 5 mM $MgCl_2$) containing 3 mg/ml lysozyme and 300 ug/ml DNAse I. The plate was incubated at 37°C for 15 min, frozen, thawed three times, and centrifuged to pellet debris. The supernatants were transferred to a new plate and tested for the presence of SEB both by ELISA and T cell hybridoma stimulation. This method produced preparations containing 0.3 and 10 ub/ml of SEB.

To produce purified mutant SEB, transformants were recovered from the 96-well plate stored at -70°C. Bacteria from overnight cultures (1 vol) containing IPTG were collected by centrifugation, resuspended in a 1:10 vol of HNM buffer containing 1-2 mg/ml lysozyme and 10 ug/ml DNAse I, and frozen and thawed three times. The suspension was centrifuged at 15,000 g for 20 min to remove bacterial debris, and the supernatant was harvested and filtered (0.2 u). The filtrate was passed through a column containing a 1:50 volume of Sepharose 4B beads coupled with 2-3 mg/ml of a mAb to SEB (B344). The beads were washed thoroughly with PBS and the toxin was eluted with 0.1 M glycine-HCl (pH 2.7) and neutralized with 1 M Na_2CO_3. The SEB was concentrated to 1 mg/ml and its buffer changed to BSS using Centricon10 microconcentrators (Amicon Corp., Denvers, MA). This method yielded 3-10 mg of toxin per liter of bacterial culture. SEB and its mutants produced in this manner were > 95% pure as judged by SDS-PAGE. Region 1 SEB mutants are listed in Table II, region 1 and 2 mutants are listed in Table III, and region 3 mutants are listed in Table IV.

The mutations described all involve a nucleotide substitution of A to G, or T to C, which would be predicted by the methodology used for their generation.

When mutations were generated using mutant oligonucleotides C or A, these mutations were concentrated in amino acids 9-23 (Region 1), or 41-53 (Region 2) of the SEB sequence (Table III).

When the oligonucleotide primer B was used, the mutants listed in Table IV were generated.

Amino acid 93 is cysteine in normal SEB. To that end, mutations in this region were not considered further because of the potential interference with disulfide binding. Thus, mutants BR-30 and BR-311 were eliminated.

Those mutants containing changes in more than one region (NOT more than one mutant), i.e., BR-474 and BA-72, were also eliminated, as was BR-267, because the toxin was highly degraded. BA-50 is a known mutant and was not studied further.

Structural Studies of SEB.

The three-dimensional crystal structure of SEB, perhaps the most widely studied member of the staphylococcal enterotoxins, has been recently reported (Swaminathan et al. (1992) Nature 359:801). A schematic drawing of SEB is shown in Figure 1. The SEB molecule contains two domains. The first is composed of residues 1-120 and the second of residues 127-239. As discussed above, three regions have been identified (Kappler et al. (1992) J. Exp. Med. 175:387) in the N-terminus part of the SEB that affect MHC class II binding and/or T cell activation. In each of the regions the specific amino acids that are responsible were determined. Some of the identified residues affect both MHC class II binding and T cell activation, whereas other affect only the latter. As superantigen-MHC class II binding is a prerequisite for T cell activation, residues affecting MHC class II binding will also influence T cell activation, thus no T cell binding information can be inferred from them. But they do provide information about MHC class II binding sites on the superantigen. On the other hand, those residues that influence T cell activation but not MHC

class II binding are likely to be in the T cell binding site on SEB.

Region 1, defined as the stretch of amino acid residues from 9-23, is bifunctional as it affects both TCR and MHC class II binding. Mutations in this region included residues in positions 10, 14, 17 and 23, as either a single or a double mutation (Table II). Asparagine at residue 23 (N23) is on the α-helix, α2, with the side chain pointed towards the solvent. It is the most important residue, being conserved among all staphylococcal enterotoxins and critical for TCR activation. Only five mutations at position 23 affected MHC class II binding, but all of them affected TCR activity. Mutations at residue 14 and 17 affected both MHC class II binding and TCR activation. Residue S14 is on a very short α-helix (α1) and is exposed to the solvent whereas F17 is located at the other end of α1. The locations of S14, F17, and N23 (Figure 1) on the surface of the toxin are favorable for making critical contacts with MHC class II molecules and/or Vβ. Residue F17 points inwards and lies in a loop sandwiched between two other loops, of residues 174-179 and 203-209. This suggests that its replacement by serine (F17S) may have introduced structural changes which reduce MHC class II binding and cytokine production. The association of S14 (on α1) with MHC class II binding, and N23 (on α2) with TCR activity, reveals the structural basis underlying the bifunctional role of region 1. Although the region consists of a small number of sequential amino acids, there are distributed on separated but adjacent elements of the secondary structure that are engaged in different functions. The proximity of α1 and α2 is consistent with the suggestion (Kappler et al. (1992) supra) that the amino acids in region 1 are situated in the trimolecular complex near the junction of Vβ and MHC class II.

Region 2 is defined as residues 40-53 and was suggested to be important in all staphylococcal enterotoxins in mediating binding to MHC class II. About half of the mutations in this region involved the conserved residue F44 (Table III). Other mutations involved residues 41, 45, 48, 52, and 53. These changes affected MHC class II binding and consequently the TCR activation. Thus, this region is probably specific for MHC class II binding. Residues 48-52 are in β-strand β2. Residue F44 is on a turn connecting β1 and β2 with the side chains exposed to solvent. It is situated favorably for engaging in critical hydrophobic binding contacts with MHC class II.

Region 3 is made up of two residues, 60 and 61, and mutation of either one affects the TCR activation but not MHC class II binding. Residues 60 and 61 are in the loop connecting β2 and β3 and are exposed to solvent (Table IV).

Example 5. Binding of Mutant SEB to HLA-DR.

Since binding to MHC class II is a prerequisite for toxin recognition by T cells, the mutations could have affected either the ability of the toxin to bind to DR molecules or the recognition of this complex by the TCR-α/β. To help distinguish these two possibilities, the HLA-DR1 homozygous lymphoblastoid line LG2 was used (Gatti and Leibold (1979) Tissue Antigens 13:35). ^{125}I-labelled LG2 cells were incubated with or without 50 ug/ml recombinant SEB for 2 hours at 37°C. A cell free lysate was prepared in 1% digitonin and incubated for 4 hours at room temperature with Sepharose beads coupled with 3 mg/ml B344 anti-SEB mAb. The beads were washed thoroughly, and the labeled bound material was analyzed by SDS-PAGE under reducing conditions (Laemmlli (1970) Nature 227:680) and autoradiography. As a control, beads bearing the anti-DR mAb, L243 (Lampson and Levy (1980) J. Immunol.

125:293) were used (1/20 the volume of lysate used with the anti-SEB beads).

SEB binds to DR molecules on LG2 (Figure 2b). Immunoaffinity-purified toxins were prepared and assessed for their ability to bind to LG2 using flow cytometry with the same anti-SEB mAb used to purify the SEB and its mutants. 3 x 10^4 LG2 cells were incubated in 100 ul of tissue culture medium overnight at 37°C with vrious concentrations of SEB or its mutants. The cells were washed thoroughly and incubated for 30 min at 4°C with approximately 1 ug/ml of the anti-SEB mAb, B344.1. The cells were washed again and incubated for 15 min at 4°C with fluoresceinated goat anti-mouse IgG1 (Fisher Scientific Co.). The cells were washed again and analyzed for surface fluorescence of the cells corrected for the fluorescence seen with the secondary reagent alone v.s the amount of toxin added. The results, shown in Figure 4, are presented for mutations in each of regions 1, 2, and 3.

The binding by four of the region 1 mutants to LG2 was indistinguishable from that of unmutated SEB. The other three mutants were reduced in their binding capacity by approximately 100-fold. These results suggest that residues between 14 and 23 within region 1 are important in MHC binding. Five of the seven mutations involved residue 23N. In only one case (BR-291, 23N→S) did this mutation reduce MHC binding. These results suggest residue 23N may be important in both MHC binding and Vβ interaction. Region 2 mutants all bound poorly to LG2, approximately 1,000 times poorer than SEB, indicating that region 2 defines a stretch of amino acids, especially 44F, important in binding of the toxin to class II MHC. Region 3 mutants were essentially unaffected in binding to LG2, strongly suggesting that this two-amino acid region (60N, 61Y) is important in Vβ interaction.

Example 5. Effect of Mutations on T Cells Bearing Different Vβ Elements.

The SEB mutants were originally identified because they stimulated either a Vβ7$^+$ or a Vβ8.3$^+$ T cell hybridoma poorly. To assess the effect of the SEB mutations on T cell recognition in more detail, the purified mutant toxins were retested at various doses on additional T cell hybridomas bearing each of the four murine Vβ elements known to recognize SEB (Vβ7, Vβ8.1-3, (White et al (1989) supra; Callahan et al. (1989) supra; Herman et al. (1991) supra). Varying concentrations of toxins were incubated at 37°C overnight with 3 x 10^4 DR$^+$ cells in 200 ul of tissue culture medium. 5 x 10^4 T cell hybridomas of requisite Vβ specificity were added in 50 ul, and the mixture incubated overnight. Response of T cell hybridomas was measured as IL-2 secreted, following Kappler et al. (1981) J. Exp. Med. 153:1198 and Mosmann (1983) J. Immunol. Meth. 65:55. The results are shown in Figures 5-7.

Among the region I mutants (Figure 5), the five involving 23N (BR-257, BR-291, BC-6, BC-66, BC-88) stimulated all of the hybridomas poorly, despite the fact that four of these bound to DR as well as unmutated SEB did. These results indicate that residue 23N is an important amino acid for Vβ interaction, but because the fifth mutant involving this amino acid, BR-291, bound poorly to MHC, this amino acid may influence MHC binding as well. The other two regions 1 mutants also stimulated poorly. In the case of BR-75, this may have been due primarily to its poor binding to DR, but the effect of the BR-210 mutation was several orders of magnitude greater on T cell stimulation than on binding to DR. Taken together, these results are evidence that during T cell recognition of SEB bound to DR, the amino acids in region 1 are situated in the trimolecular

complex at the junction between Vβ and MHC, so that individual residues may interact with either component.

The mutations in the other regions produced less complicated phenotypes. All of the region 2 mutants were defective in stimulation of all of the T cell hybridomas, regardless of the Vβ element in their receptors (Figure 6). There were small differences, but in general the effect of mutations on stimulation was about the same as that seen on DR binding. These results were consitent with the conclusion that mutations in region 2 primarily affect DR binding.

The two-amino acid region 3 mutants were the most discriminating (Figure 7). Despite the fact that random mutants in a 20-amino acid strech flanking this region were generated, all mutations affecting function were found in these two amino acids. These mutants failed to stimulate the hybridomas bearing Vβ7 and Vβ8.1, but not Vβ8.2 or Vβ8.3. To insure that this property was not peculiar to these particular hybridomas, the toxins were tested with four other T cell hybridomas: one $V\beta7^+$. two $V\beta8.1^+$, and one $V\beta8.3^+$. The results were indistinguishable from those in Figure 7 (data not shown).

Example 6. Requirement for T Cell Interaction for In Vivo Effects of SEB.

The question of how important the superantigen properties of the bacterial toxins are to their in vivo toxic effects is unresolved. Previous experiments by the inventors suggested that the toxicity of SEB in mice was related to its ability to stimulate T cells in a Vβ-specific manner, since the toxic effect of SEB was directly related to the frequency of T cells bearing the relevant Vβ elements (Marrack et al. (1990) J. Exp. Med. 171:455). However, the ability of some of S. aureus toxins to bind to class II on monocytes and stimulate the production of cytokines such as TNF and

IL-1 (Parsonnet (1989) Rev. Infect. Dis. 1:263) opens the possibility that direct monocyte stimulation may be sufficient to account for much of the toxin pathology in some situations.

To test this idea, mice were injected with various concentrations of region I mutant BR-257, which binds very well to class II MHC but does not stimulate T cells except at extremely high levels. Unmutated SEB and mutant BR-358, which like all of the region 2 mutants binds very poorly to class II MHC, were used as controls. To minimize the effects of LPS, which might contaminate the preparations, C3H/HeJ mice were used, a strain defective in LPS responsiveness. Since rapid weight loss is one of the most obvious immediate toxic effects of SEB in mice (Marrack et al. (1990) supra), the mice were weighed daily after the injection on day 0.

Groups of three mice were weighed and then given balanced salt solution (BSS) containing either nothing, 50 ug, or 100 ug of recombinant SEB, mutant SEB BR-257, or mutant SEB BR-358. The mice were weighed daily at the same time of day until they died. The results are shown in Figure 8. Results are presented as the average percent change from the starting weight for the surviving mice.

Mice given either 50 or 100 ug of recombinant SEB lost weight rapidly over 3-4 days, and all of the mice were dead by day 5. Mice given mutant BR-358 showed no effects and were indistinguishable from those given BSS alone. Mice given 50 ug of BR-257 were unaffected as well; however, those given 100 ug of BR-257 showed a slight weight loss followed by recovery.

These results confirm that in mice the majority of the toxicity of SEB is dependent on its ability to stimulate T cells, suggesting that T cell-derived lymphokines themselves or those produced by other cells activated by T cells are very important in the mode of

action of this toxin. However, the small effect of BR-257 at the higher dose raises the possibility of a contribution from class II-bearing cells directly stimulated by bound SEB without T cell involvement.

Example 7. Protective Effect of SEB Mutants.

The protective effect of SEB mutants was tested. In these experiments, mice received doses of saline solution or 100 ug BR-257 three months prior to a challenge with wild-type SEB. On the day of the challenge (day "0"), the mice received 50 ug of SEB intraperitoneal. Weight change and survival were measured. Results are shown in Figure 9.

All mice which had received the control died 4-5 days after challenge with SEB, whereas there was a protective effect shown in the mice which had been immunized with the SEB mutant.

Example 8. Production of SEA Mutants and Their Protective Effects in Animals.

Staphylococcal enterotoxin A (SEA) mutants were produced according to the procedures described above. Superimposing the amino acid sequence of SEA on that of SEB, it has been bound that a mutation at position 45 inhibits SEA's ability to bind to MHC, in a similar manner to that observed with the position 45 SEB mutant.

Similar studies were conducted with primates. Monkeys received either wild-type SEB, or either of the reion 1 SEB mutants BR-257 (mutated at F44) or BR-358 (mutated at N23), and the induction of an emetic response assessed. Both mutant SEB molecules were either ineffective or much less effective in inducing an emetic response in primates, than wild-type SEB.

These results confirm that the method of producing mutant superantigen described in this disclosure is applicable generally to all superantigens, and provides

a method of protecting patients from the pathological effect of superantigens.

TABLE I. KNOWN SUPERANTIGEN SEQUENCES AND STRUCTURES

Staphylococcus	Staphylococcal enterotoxin A	Huang et al (1987) J. Biol. Chem. 262:7006 Betley et al. (1988) J. Bacteriol. 170:34
	Staphylococcal enterotoxin B	Jones & Khan (1986) J. Bacteriol. 166:29 Huang & Bergdoll (1970) J. Biol. Chem. 245:3518 Ranelli et al. (1985) Proc. Natl. Acad. Sci. 82:5850
	Staphylococcal enterotoxin C1 and C3	Schmidt & Spero (1983) J. Biol. Chem. 258:6300 Bohach & Schlievert (1987) Mol. Gen. Genet. 209:15 Couch & Betley (1989) J. Bacteriol. 171:4507
	Staphylococcal enterotoxin D	Bayles & Iandolo (1989) J. Bacteriol. 171:4799
	Staphylococcal enterotoxin E	Couch et al. (1989) J. Bacteriol. 170:2954
Toxic Shock Toxin		Schlievert et al. (1981) J. Infect. Dis. 143:509 Blomster-Hautamaa et al. (1986) J. Biol. Chem. 261:15783 Bergdoll et al. (1981) Lancet 1:1017
Exfoliating Toxins		Lee et al. (1987) J. Bacteriol. 169:3904
Streptococcus	Streptococcal pyrogenic toxin C	Goshorn & Schlievert (1988) Infect. Immun. 56:2518 Tomai et al. (1990) J. Exp. Med. 172:359
Mouse Mammary Tumor Virus		Fasel et al. (1982) EMBO J. 1:3 Donehower et al. (1981) J. Virol. 37:226 Donehower et al. (1983) J. Virol. 45:941 Raceviskis & Prakash (1984) J. Virol. 51:604 Choi et al. (1991) Nature 350:203 Acha-Orbea et al. (1991) Nature 350:207 Pullen et al. (1992) J. Exp. Med. 175:41 Moore et al. (1987) J. of Virology 61:480

TABLE II. REGION 1 SEB MUTANTS.

Mutant Name	Position	Change(s)
BR-75	F17	Phe-Ser
BR-210	S14	Ser-Leu
BR-257	10, N23	Asp-Asn; Asn-Asp
BR-291	N23	Asn-Ser
BR-358	F44	Phe-Ser
BR-374	D48, 160	Asp-Gly; Leu-Val
BR-30	Y91	Tyr-Cys
BR-311	C93	Cys-Arg
BR-474	46, C93	Tyr-Ser; Cys-Arg
BR-267	F44, 54, 55	Phe-Ser; Lys-Arg; Asp-Val

TABLE III. REGION 1 AND 2 SEB MUTANTS GENERATED WITH MUTANT OLIGONUCLEOTIDES A OR C.

Mutant Name	Position	Change(s)
BC-6	N23	Asn-Ile
BC-66	N23	Asn-Tyr
BC-88	N23	Asn-Lys
BA-3	F44	Phe-Cys
BA-15	L45	Leu-Val
BA-24	41, 53	Ile-Arg; Gln-Val
BA-31	46, 52	Tyr-Leu; Ser-Phe
BA-50	F44	Phe-Ser
BA-53	F44, 43	Phe-Leu; Ile-Arg
BA-62	Y61, 189	Gln-Ser; Ile-Arg
BA-72	L45, N60	Leu-Tyr; Asn-Lys

TABLE IV. REGION 3 SEB MUTANTS

Mutant Name	Position	Change(s)
BB-14	36, Y61	Gln-Leu; Tyr-Cys
BB-21	N60	Gln-Asn
BB-47	Y61	Tyr-Gln

CLAIMS

1. A method for preventing the toxic effects of a superantigen by treatment with a molecule, wherein said molecule elicits antibody production without inducing T cell activation.

2. The method of claim 1 wherein said molecule is a mutated superantigen.

3. The method of claim 1 wherein said molecule is a modified superantigen.

4. A molecule comprising a mutated superantigen.

5. A molecule comprising a modified superantigen.

6. A method of modifying T cell response elicited by an antigen comprising administering a molecule which interacts with specific Vβ elements of T cell receptors (TCR).

7. The method of claim 6 wherein said molecule is a mutated superantigen.

8. The method of claim 6 wherein said molecule is a modified superantigen.

9. A method for treating the toxic effects of superantigen by treatment with a molecule, wherein said molecule elicits antibody production without inducing T cell activation.

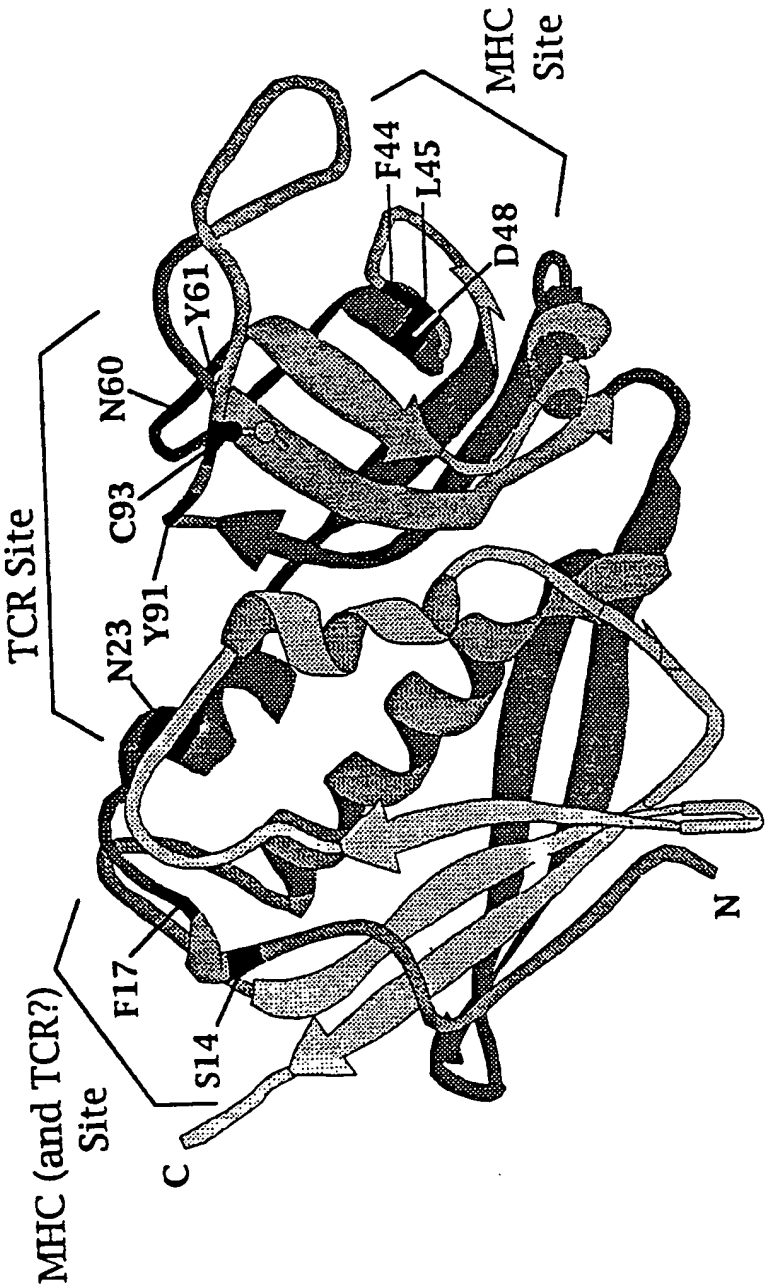

MHC Site
F44
L45
D48
Y61
N60
C93
Y91
N23
TCR Site
F17
S14
MHC (and TCR?) Site
C
N

FIG. 1

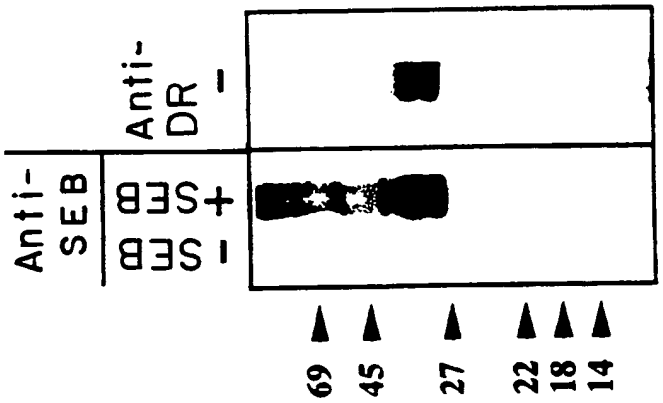

Anti-DR
Anti-SEB
+SEB
-SEB
69
45
27
22
18
14

FIG. 2B

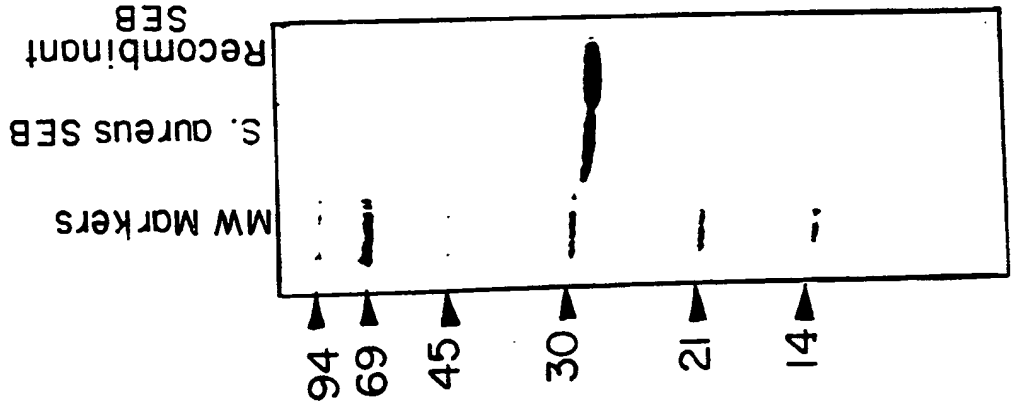
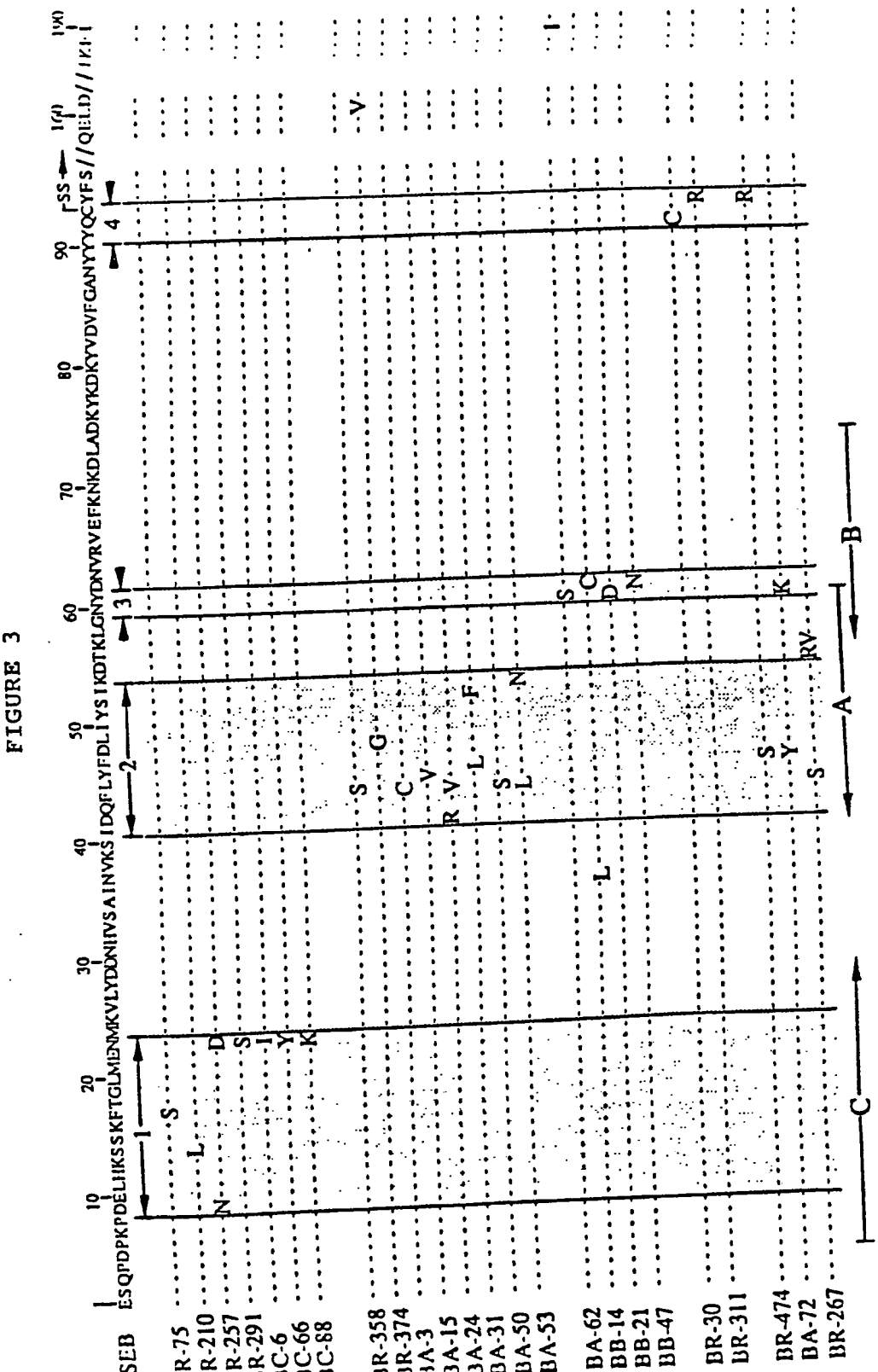

Recombinant SEB
S. aureus SEB
MW Markers
94
69
45
30
21
14

FIG. 2A

FIGURE 3


SEB ESQPDPKPDELHKSSKFTGLMENMKVLYDDNHVSAINVKSIDQFLYFDLIYSIKDTKLGNYDNVRVEFKNKDLADKYKDKYVDVFGANYYYQCYFSKTIN
BR-75
BR-210
BR-257
BR-291
BC-6
BC-66
BC-88
BR-358
BR-374
BA-3
BA-15
BA-24
BA-31
BA-50
BA-53
BA-62
BB-14
BB-21
BB-47
BR-30
BR-311
BR-474
BA-72
BR-267
1
2
3
4
A
B
C

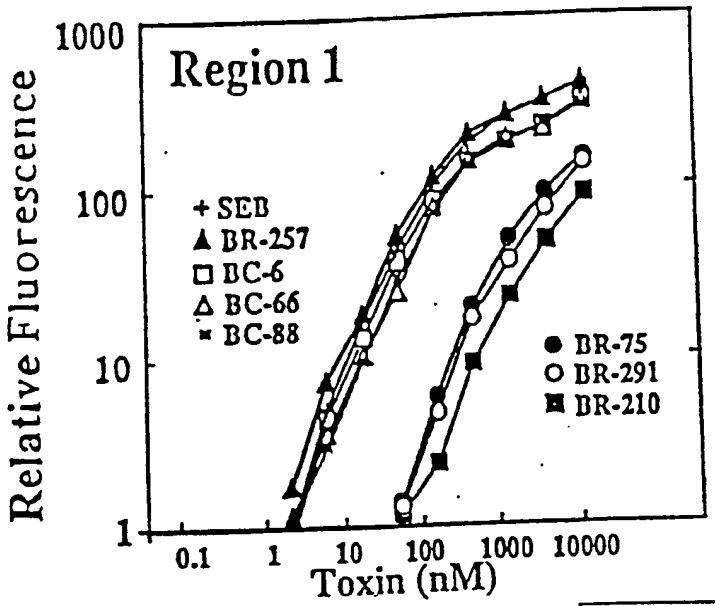


FIG.4A

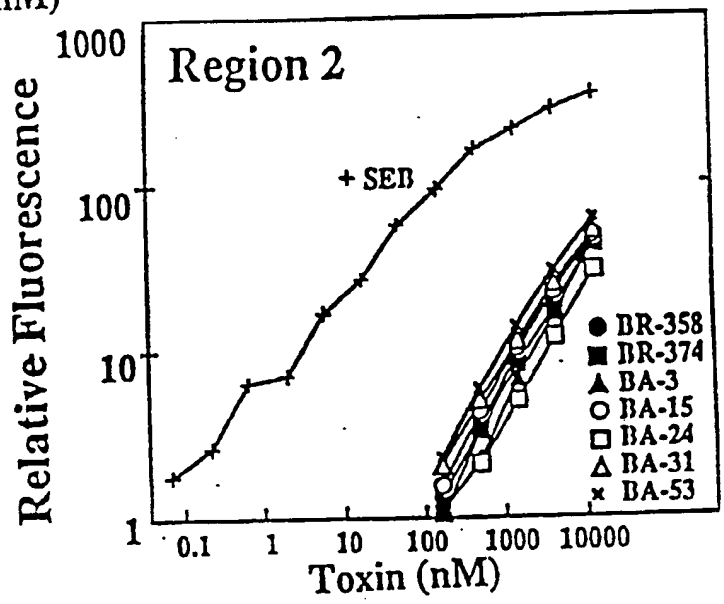


FIG.4B

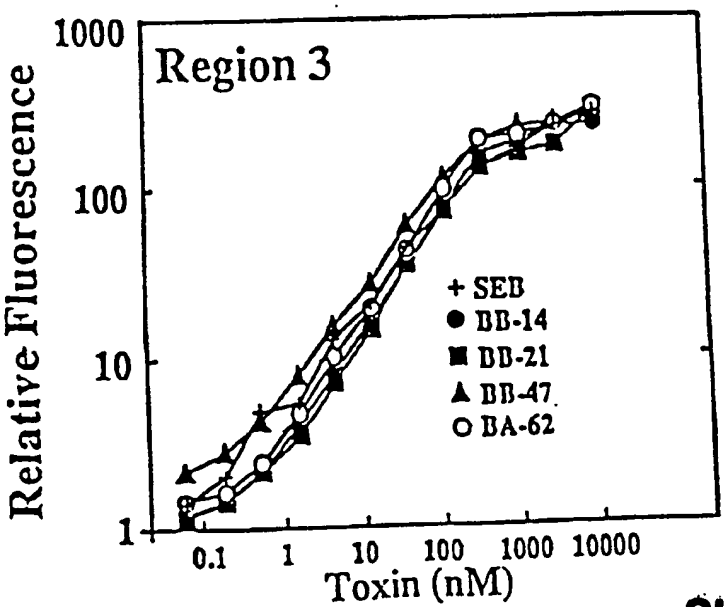


FIG.4C

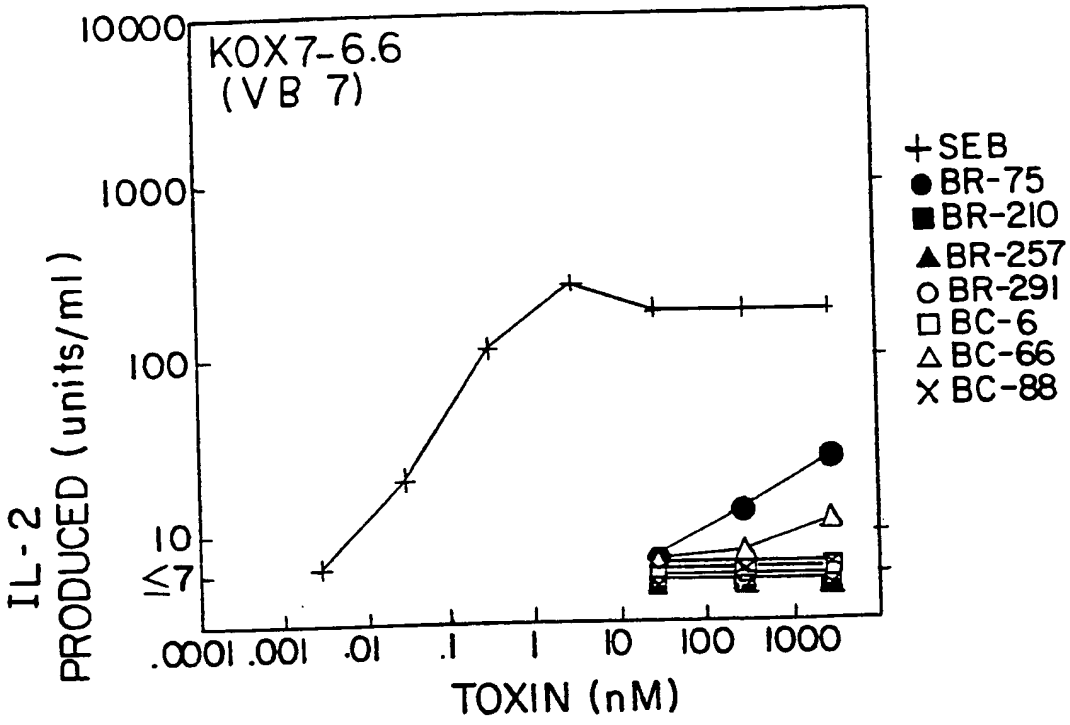

KOX7-6.6
(VB 7)
IL-2 PRODUCED (units/ml)
10000
1000
100
10
≤7
.0001 .001 .01 .1 1 10 100 1000
TOXIN (nM)
+SEB
●BR-75
■BR-210
▲BR-257
○BR-291
□BC-6
△BC-66
×BC-88

FIG.5A

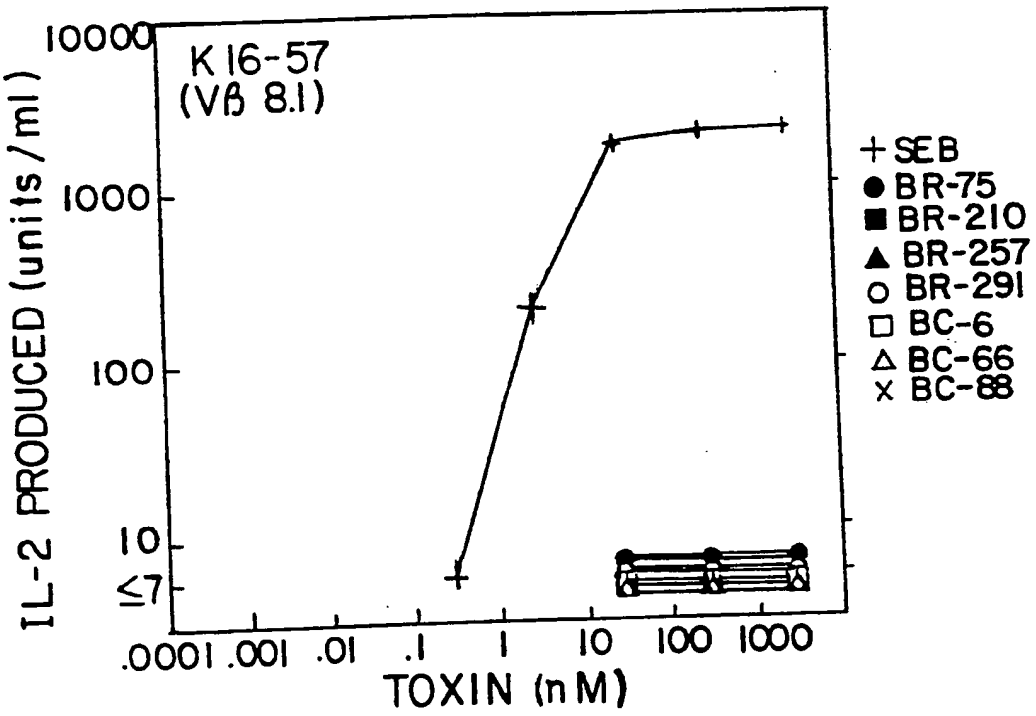

K16-57
(Vβ 8.1)
IL-2 PRODUCED (units/ml)
10000
1000
100
10
≤7
.0001 .001 .01 .1 1 10 100 1000
TOXIN (nM)
+SEB
●BR-75
■BR-210
▲BR-257
○BR-291
□BC-6
△BC-66
×BC-88

FIG.5B

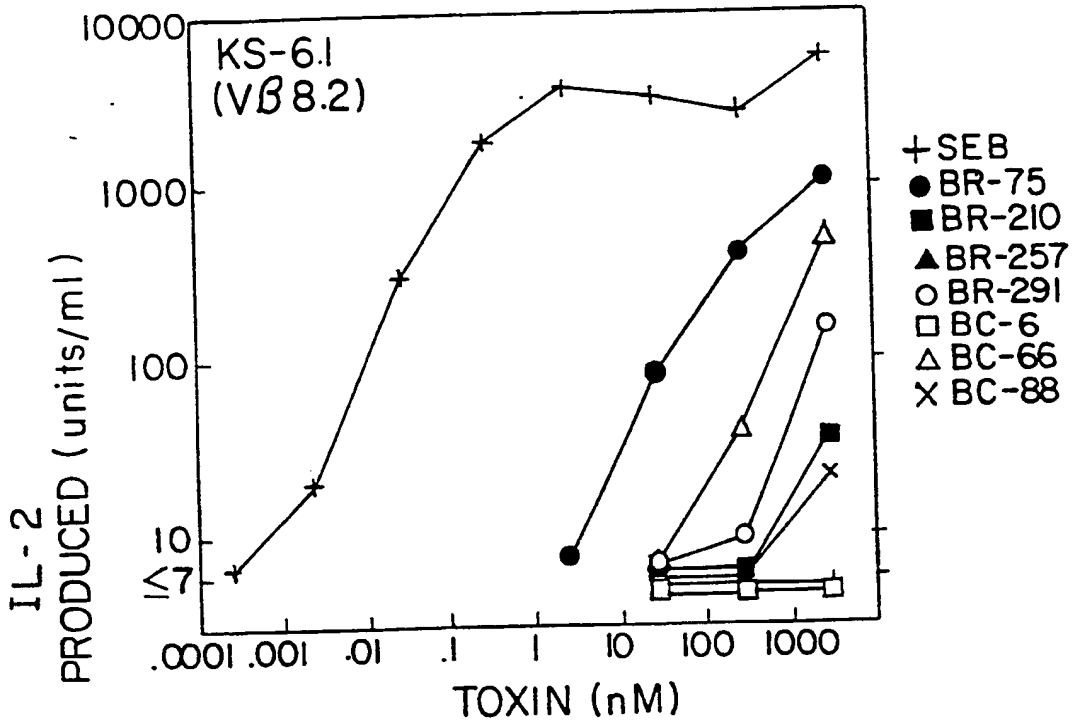

KS-6.1
(Vβ 8.2)
IL-2 PRODUCED (units/ml)
10000
1000
100
10
≤7
.0001 .001 .01 .1 1 10 100 1000
TOXIN (nM)
+SEB
●BR-75
■BR-210
▲BR-257
○BR-291
□BC-6
△BC-66
×BC-88

FIG.5C

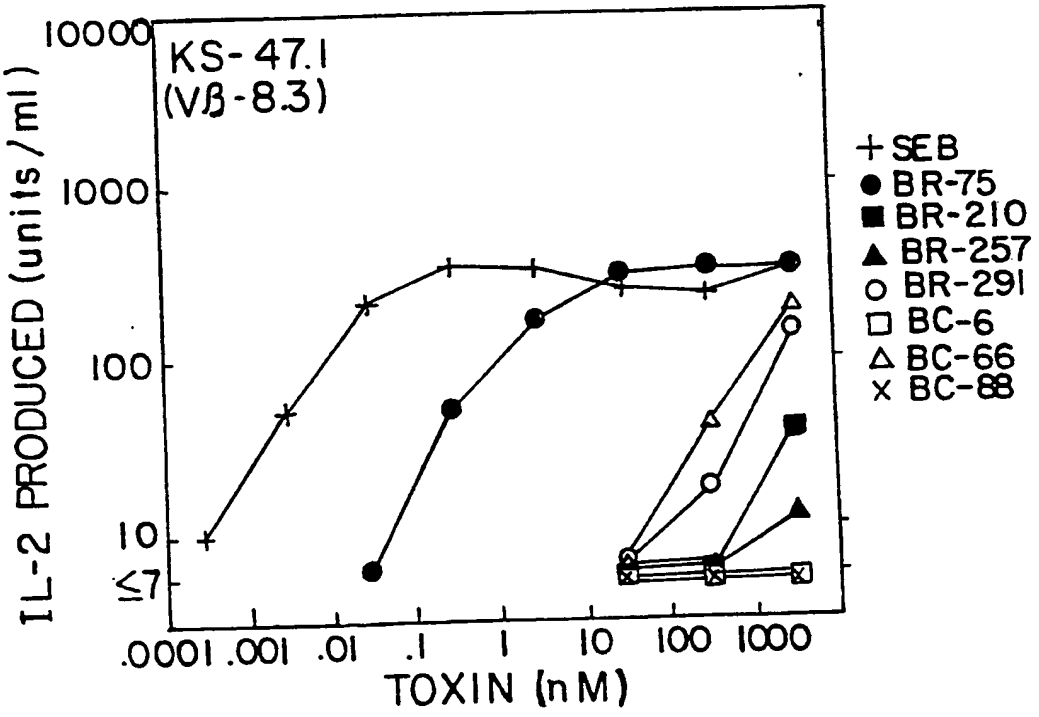

KS-47.1
(Vβ-8.3)
IL-2 PRODUCED (units/ml)
10000
1000
100
10
≤7
.0001 .001 .01 .1 1 10 100 1000
TOXIN (nM)
+SEB
●BR-75
■BR-210
▲BR-257
○BR-291
□BC-6
△BC-66
×BC-88

FIG.5D

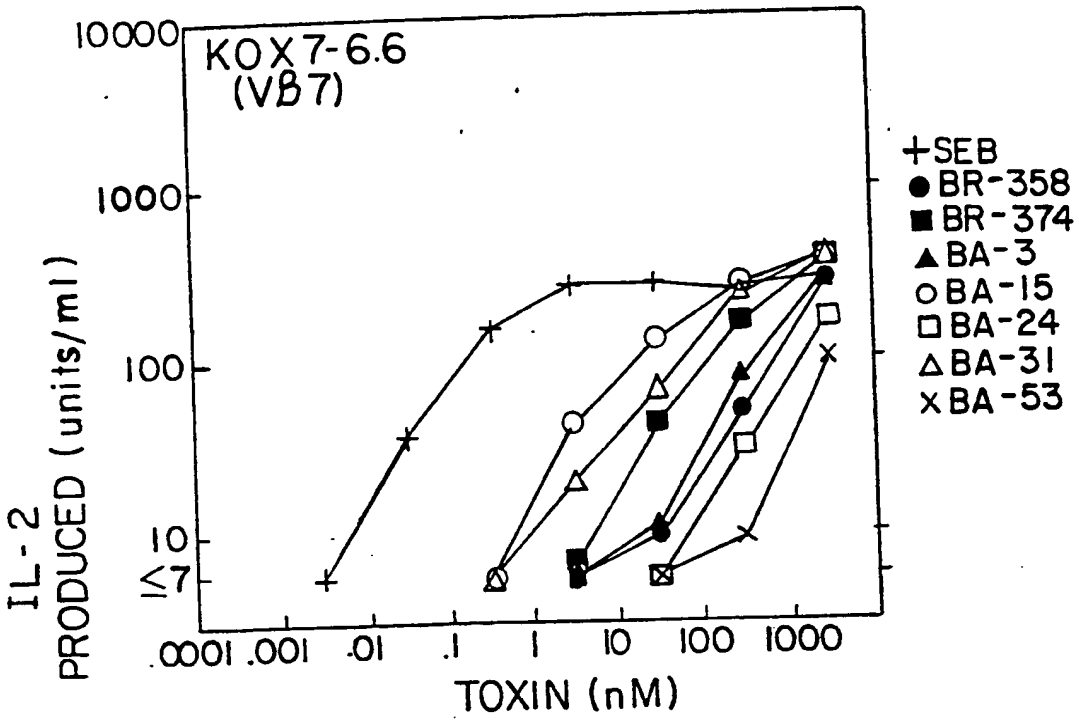

KOX7-6.6
(Vβ7)
10000
1000
100
10
≤7
IL-2 PRODUCED (units/ml)
.0001 .001 .01 .1 1 10 100 1000
TOXIN (nM)
+SEB
●BR-358
■BR-374
▲BA-3
○BA-15
□BA-24
△BA-31
×BA-53

FIG. 6A

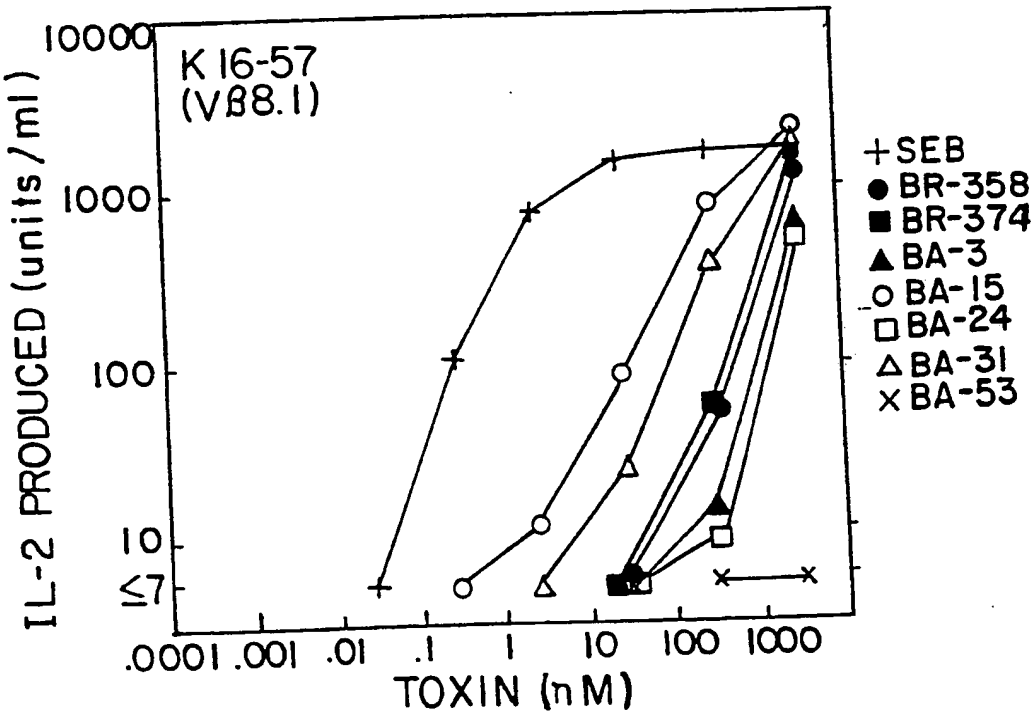

K16-57
(Vβ8.1)
10000
1000
100
10
≤7
IL-2 PRODUCED (units/ml)
.0001 .001 .01 .1 1 10 100 1000
TOXIN (nM)
+SEB
●BR-358
■BR-374
▲BA-3
○BA-15
□BA-24
△BA-31
×BA-53

FIG. 6B

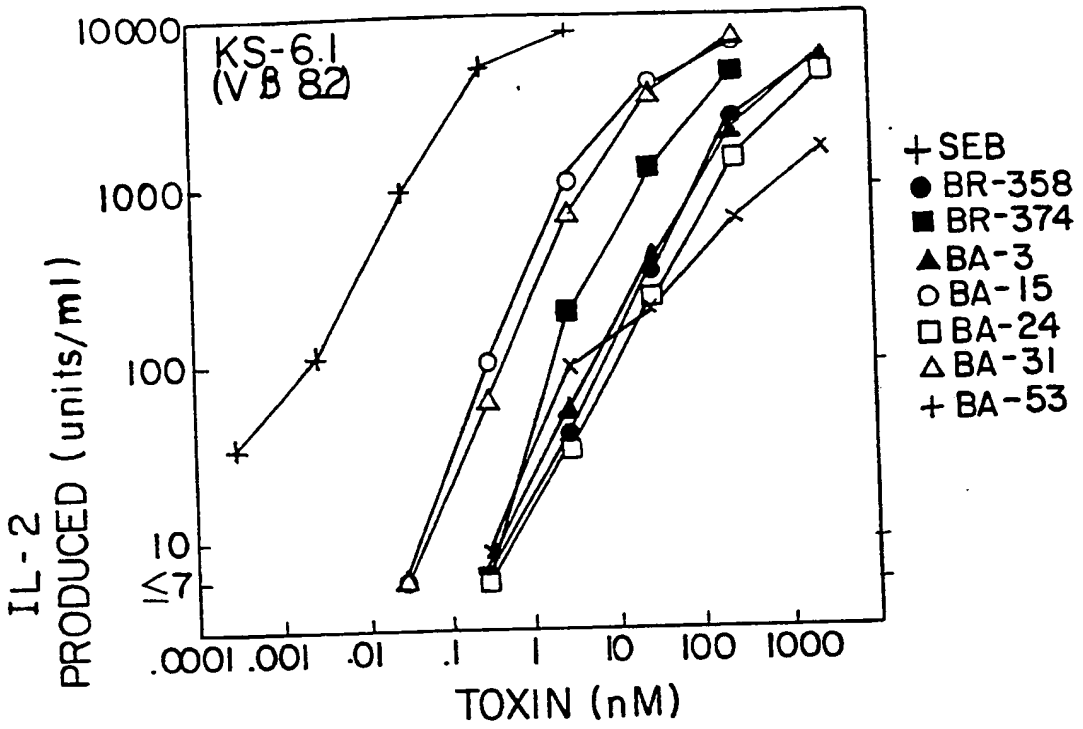

KS-6.1
(Vβ 8.2)
IL-2 PRODUCED (units/ml)
10000
1000
100
10
≤7
.0001 .001 .01 .1 1 10 100 1000
TOXIN (nM)
+SEB
● BR-358
■ BR-374
▲ BA-3
○ BA-15
□ BA-24
△ BA-31
+ BA-53

FIG. 6C

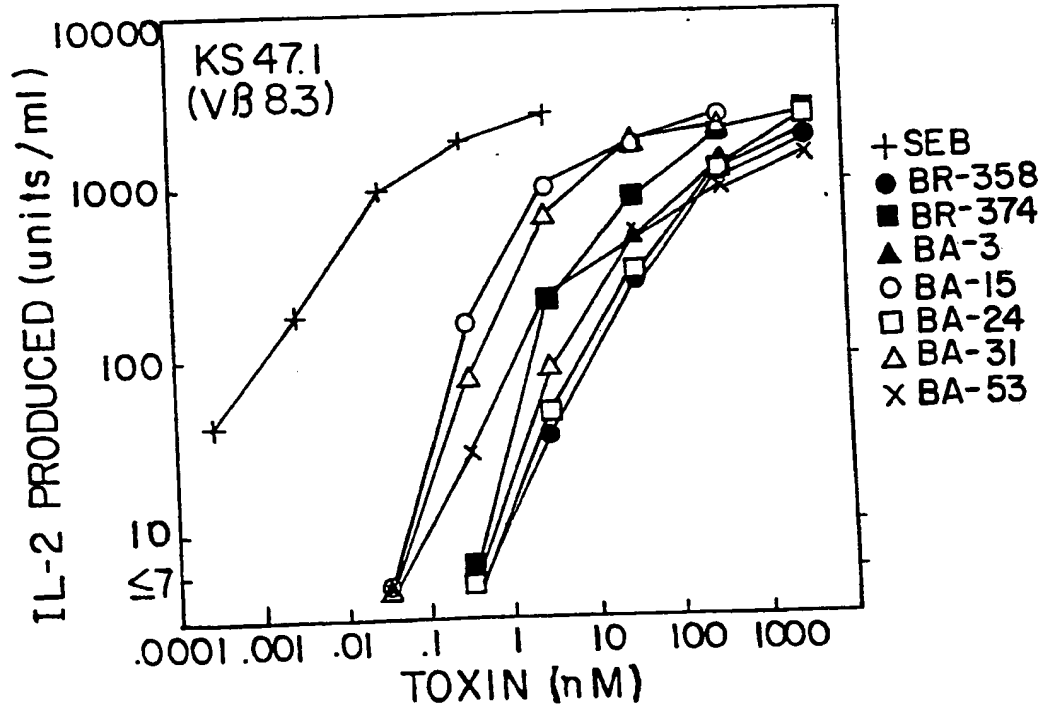

KS 47.1
(Vβ 8.3)
IL-2 PRODUCED (units/ml)
10000
1000
100
10
≤7
.0001 .001 .01 .1 1 10 100 1000
TOXIN (nM)
+SEB
● BR-358
■ BR-374
▲ BA-3
○ BA-15
□ BA-24
△ BA-31
× BA-53

FIG. 6D

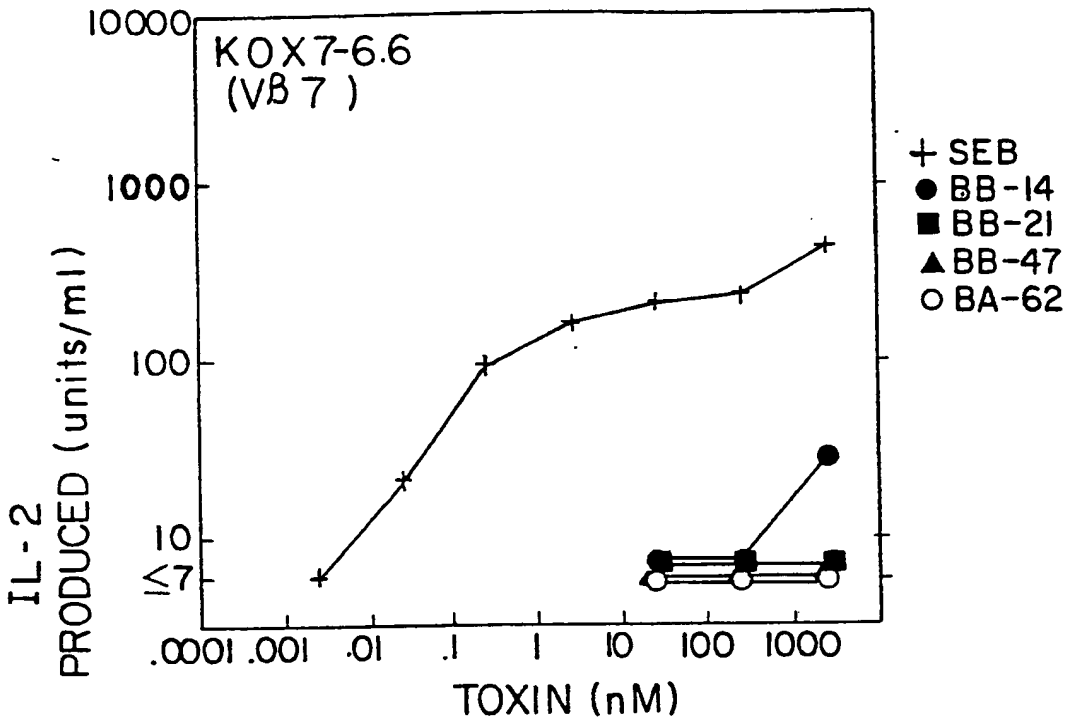

10000
1000
100
10
≤7
KOX7-6.6
(Vβ 7)
IL-2 PRODUCED (units/ml)
.0001 .001 .01 .1 1 10 100 1000
TOXIN (nM)
+ SEB
● BB-14
■ BB-21
▲ BB-47
○ BA-62

FIG. 7A

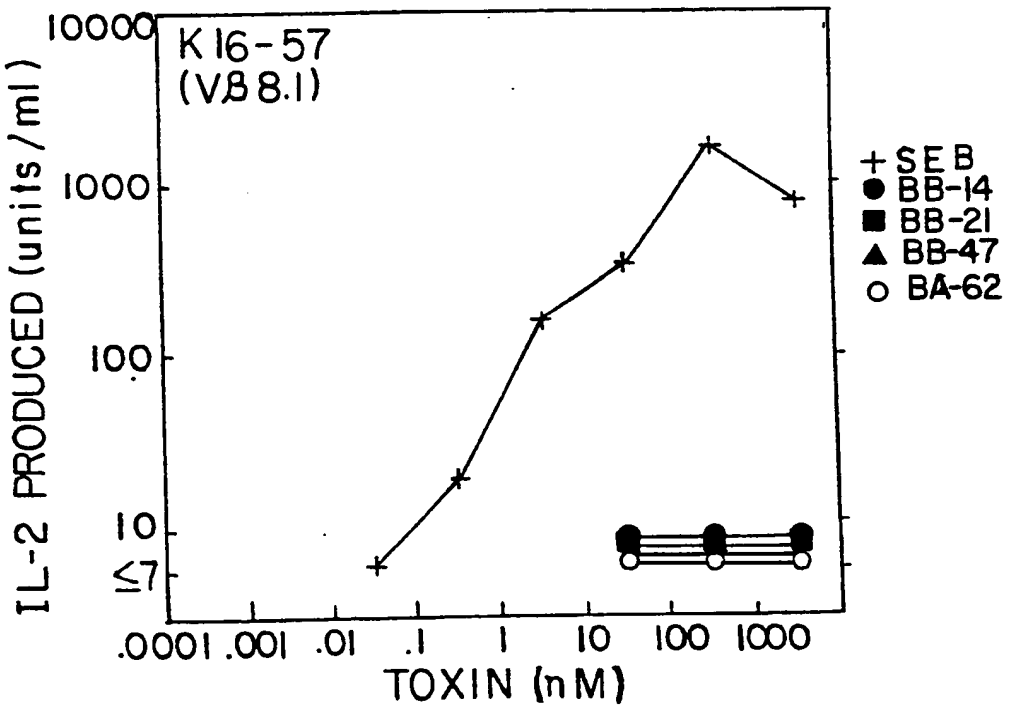

10000
1000
100
10
≤7
K16-57
(Vβ 8.1)
IL-2 PRODUCED (units/ml)
.0001 .001 .01 .1 1 10 100 1000
TOXIN (nM)
+ SEB
● BB-14
■ BB-21
▲ BB-47
○ BA-62

FIG. 7B

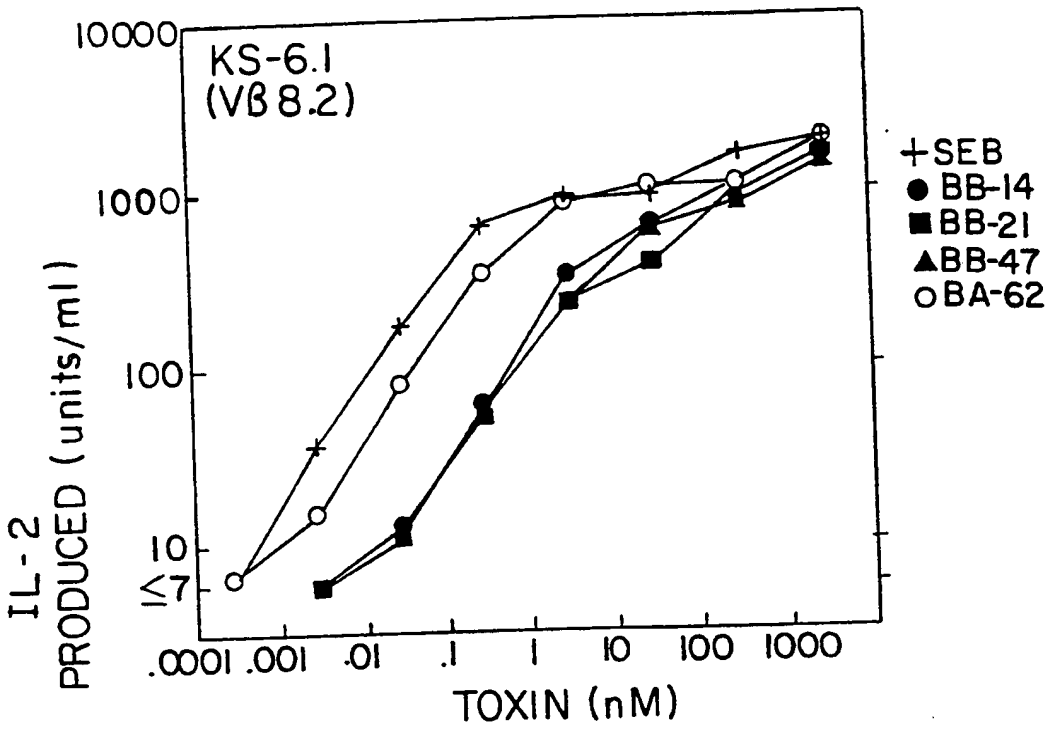

KS-6.1
(Vβ 8.2)
10000
1000
100
10
≤7
IL-2 PRODUCED (units/ml)
.0001 .001 .01 .1 1 10 100 1000
TOXIN (nM)
+SEB
● BB-14
■ BB-21
▲ BB-47
○ BA-62

FIG.7C

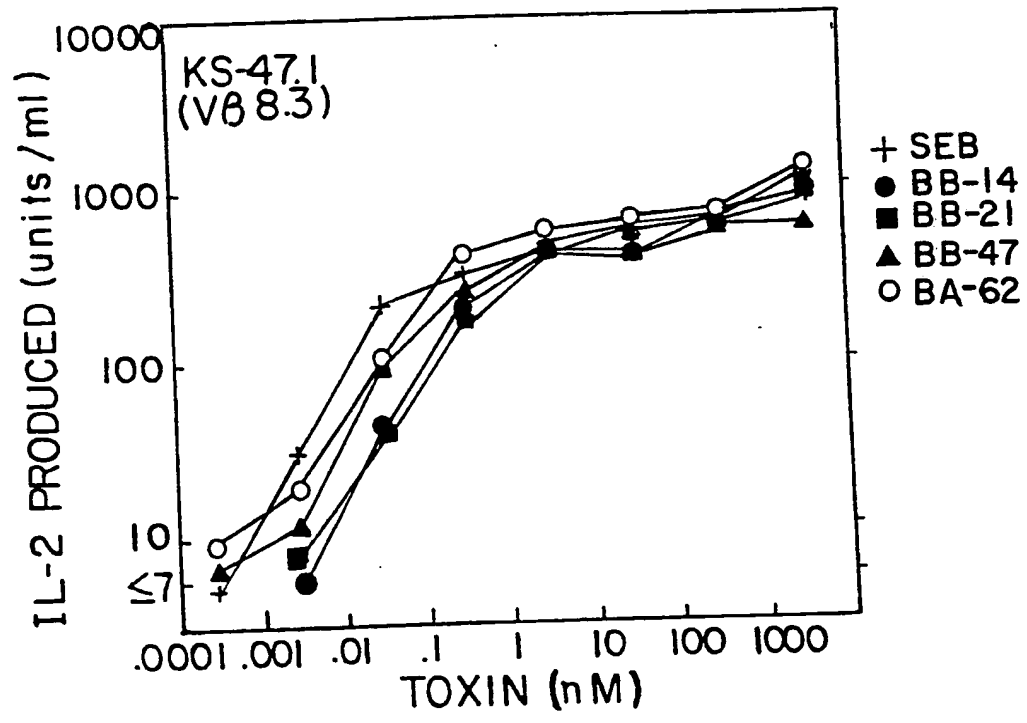

KS-47.1
(Vβ 8.3)
10000
1000
100
10
≤7
IL-2 PRODUCED (units/ml)
.0001 .001 .01 .1 1 10 100 1000
TOXIN (nM)
+ SEB
● BB-14
■ BB-21
▲ BB-47
○ BA-62

FIG.7D

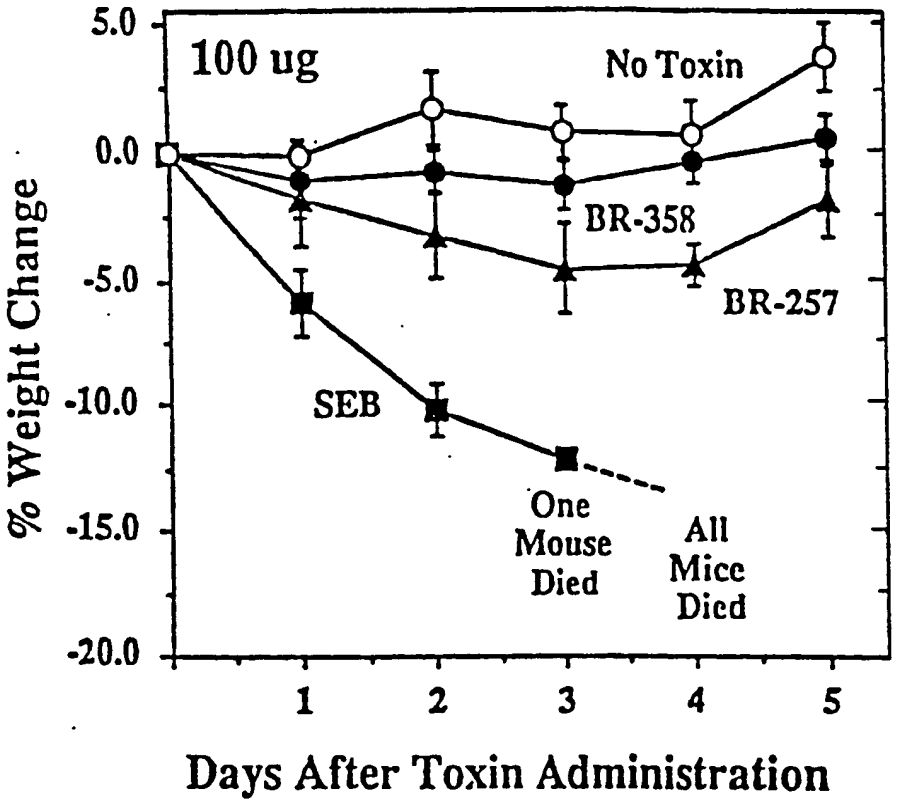

100 ug
No Toxin
BR-358
BR-257
SEB
One Mouse Died
All Mice Died
% Weight Change
5.0
0.0
-5.0
-10.0
-15.0
-20.0
1
2
3
4
5
Days After Toxin Administration

FIG.8A

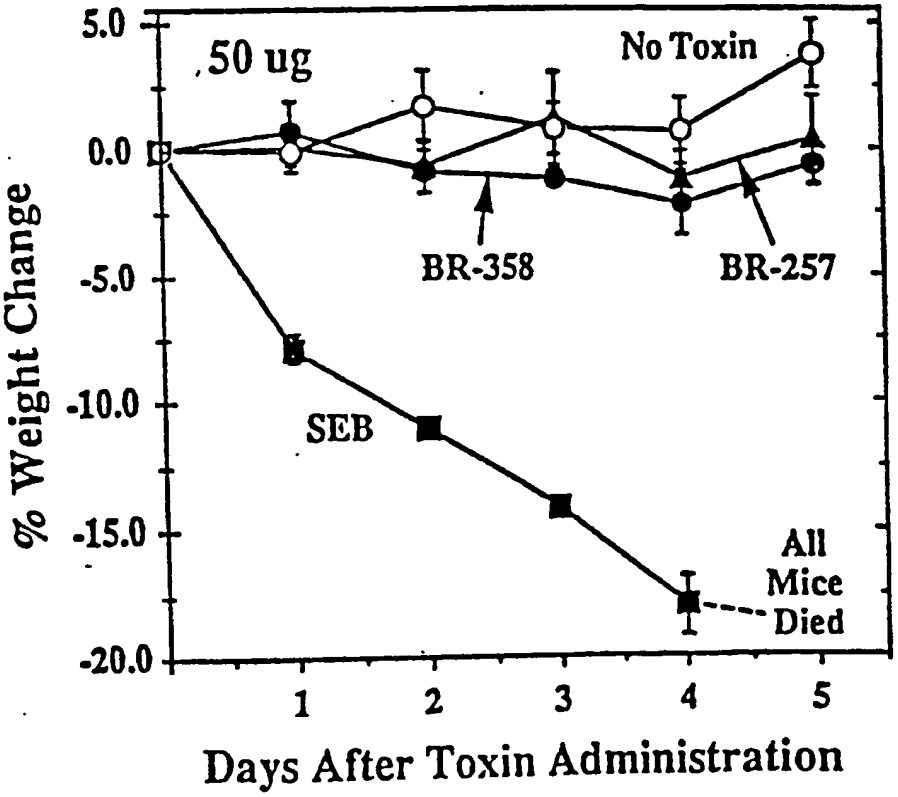
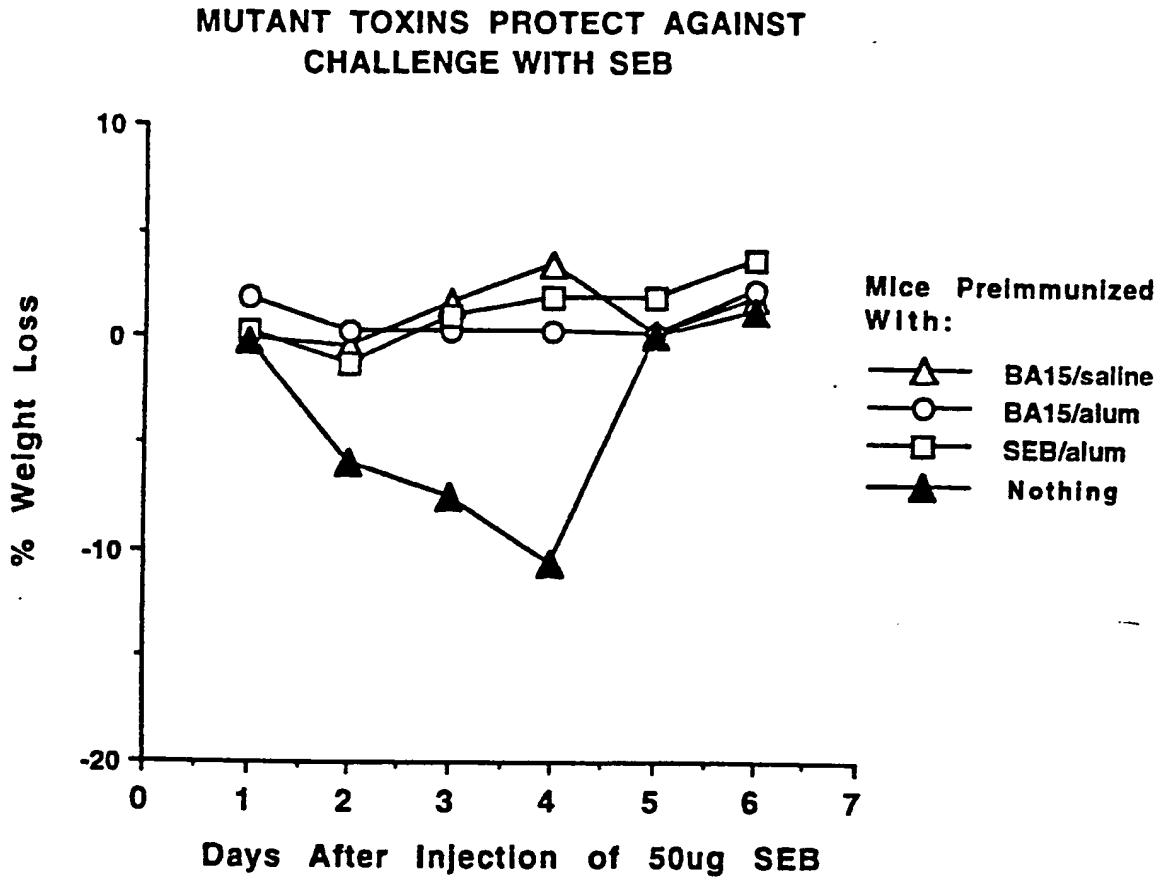

50 ug
No Toxin
BR-358
BR-257
SEB
All Mice Died
% Weight Change
5.0
0.0
-5.0
-10.0
-15.0
-20.0
1
2
3
4
5
Days After Toxin Administration

FIG.8B

FIGURE 9


MUTANT TOXINS PROTECT AGAINST CHALLENGE WITH SEB
% Weight Loss
10
0
-10
-20
0
1
2
3
4
5
6
7
Days After Injection of 50ug SEB
Mice Preimmunized With:
BA15/saline
BA15/alum
SEB/alum
Nothing

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/00839

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/92; 435/7.1, 7.2, 7.33, 69.1, 69.3, 71.1, 172.3, 240.2, 320.1; 514/2; 530/350; 536/22.1, 23.1, 23.2, 23.4, 23.7

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, BIOSIS
search terms: superantigen#, SEB, staphylo?, vbeta, enterotoxin, enterotoxin b

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Jour. Exp. Med., Volume 171, issued February 1990, P. Marrack et al., "The Toxicity of Staphylococcal Enterotoxin B in Mice is Mediated by T Cells", pages 455-464., see entire document.	1-9
Y	D. M. Glover, "Gene Cloning", published 1984, by Chapman and Hall (N.Y.), see pages 20-47.	1-9
Y	Jour. Bact., Volume 166, No. 1, issued April 1986, C. L. Jones et al, "Nucleotide Sequence of the Enterotoxin B Gene from Staphylococcus aureus", pages 29-33, see entire document.	1-9

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search
27 April 1993

Date of mailing of the international search report
04MAY 1993

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231
Facsimile No. NOT APPLICABLE

Authorized officer
ROBERT A. WAX
Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/00839

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (5):

A01 N 37/18, A61K 37/00, 39/02; C07K 3/00, 13/00, 15/00, 17/00; C12N 5/00, 15/00; C12P 21/04, 21/06; C12Q 1/00;

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

424/92; 435/7.1, 7.2, 7.33, 69.1, 69.3, 320.1; 514/2; 530/350; 536/22.1, 23.1, 23.2, 23.4, 23.7